PARASITOLOGY

PARASITOLOGY

(Founded by G. H. F. Nuttall, F.R.S.)

EDITED BY

D. KEILIN and P. TATE

IN CONJUNCTION WITH

A. BISHOP
F. J. BROWN
P. A. BUXTON
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PLASMODIUM GALLINACEUM IN TISSUE CULTURE. OBSERVATIONS AFTER ONE YEAR OF CULTIVATION

BY M. XAVIER DE OLIVEIRA AND H. MEYER

Instituto de Biofisica, Rio de Janeiro

(With Plate I)

In our previous papers, the cultivation of the exoerythrocytic form of *Plasmodium gallinaceum* has been described (Meyer & de Oliveira, 1947; Meyer, 1949*a*; de Oliveira, 1950). The development of this parasite in tissue culture has also been obtained by Hawking (1946), Lewert (1950) and others. Whereas Hawking used for this purpose the method of Carrel flasks and hanging drops, Lewert cultivated the parasites in roller tubes and succeeded in maintaining them in tissue cultures for a long period (90 days), after which he could infect chicken with his cultures. He was, however, unable to see the parasites in these cultures, either in the living cells, or in fixed and stained preparations.

We have cultivated the exoerythrocytic form of *P. gallinaceum* in Carrel flasks, changing to hanging drops and back to Carrel flasks again, and could observe the parasite both *in vivo* and also in fixed and stained cultures, during a period of 5 months. However, it was not possible with this method to obtain large quantities of infected cultures, which would allow a detailed study of the life cycle of the parasite in the cultures.

The following is a description of the method and the results recently obtained from the cultivation of *P. gallinaceum* in roller tubes, as developed by Gey & Gey (1936). This method produces a great quantity of tissue cells and proved useful in the cultivation of *P. gallinaceum* (Lewert, 1950).

MATERIAL AND METHODS

The cultivation in roller tubes was begun with fragments of brain from chickens with exoerythrocytic infection. The culture medium was composed of chick plasma diluted 1:1 with Tyrode or Ringer solution and with just enough chick embryo extract to permit the clotting of the plasma. The supernatant fluid was a mixture of 1 part chick serum and 1 part Tyrode solution with diluted chick embryo extract, in proportions of 5:1. The supernatant fluid was renewed twice weekly, after a 'patch' with chick plasma. After 1–2 weeks of cultivation in one tube, the cultures were transferred to hanging drops for microscopic examination at high magnification, and for fixation and staining of some cultures with Giemsa.

When cultures showed the presence of many parasites, fresh tissue was added and placed at the side of the infected tissue at the next transfer. Generally embryonic chicken brain was used, but embryonic spleen and liver also gave good results. After one passage, together with fresh tissue, in hanging drop, the cultures were transferred to roller tubes again, where they remained for 10–14 days, depending upon the grade of infection and the general appearance of the tissue

cells. After this period, the routine transfer to hanging drops and the addition of fresh tissue, when necessary, was repeated periodically.

With the treatment described above it has been possible to maintain *P. gallinaceum* for 1 year *in vitro*. The parasites proliferate actively in the cells of embryonic brain, liver and spleen. They pass from one infected tissue to another, and a single infected culture, when injected intramuscularly into a chick, gives rise to an exoerythrocytic infection. Later, the infection passes into the blood, as has been previously described (Meyer, 1949*a*).

In our previous experiments, as in the cultures of Hawking, the infection was always limited to a restricted area of the tissue culture. We observed that the newly formed merozoites are in active motion for only a very short time after having left the host cell, and are removed by the phagocytic action of the neighbouring cells. If, owing to lack of available cells, they have to remain free, they quickly degenerate.

In the roller tubes the liquid phase is in constant movement. This seems to facilitate the transport of parasitized cells or even of free parasites to regions distant from the original point of infection. After cultivation in roller tubes for a short period, great areas of the tissue cultures are infected with parasites. This fact, and the luxurious growth of the cells in roller tubes, makes possible a great number of subcultures, and it has thus been possible to undertake a series of experiments for the study of the life cycle of *P. gallinaceum* and its relation to the host cell, as we have previously reported for *Trypanosoma cruzi* and *Toxoplasma*.

OBSERVATIONS *IN VIVO* OF THE VARIOUS PHASES OF THE CYCLE

The roller tube cultures of *P. gallinaceum* show the same high grade infection as has been observed by us with *Toxoplasma* or *Trypanosoma cruzi* in tissue cultures (Meyer & de Oliveira 1945, 1948) (Pl. I, fig. 1). Frequently cells can be found completely filled with a very large number of parasites that can be likened to the masses which fill the endothelial cells in the brain capillaries of infected chicken. The parasitized cells can undergo mitosis, as in *T. cruzi* or *Toxoplasma*. Most of our *in vivo* observations were of the final phase of the cycle, because these forms are much easier to recognize than the initial ones. We could follow the development of small homogeneous round bodies which correspond to the young schizonts in Giemsa-stained preparations, until they became typical segmenters (or Rosaceas) as shown in Pl. I, fig. 2 (Meyer, 1949*b*). In other observations, great schizonts which present themselves as homogeneous masses in the cells develop gradually into more than one schizont. An intense movement of the already segmented parasites can be observed, which stops a short time before the rupture of the host cell. The cell seems to remain in good condition even when the development of the parasite is far advanced. The development is not determined by the host cell, but seems to follow an independent periodicity, since frequently cells can be found with only one schizont that ruptures and frees the parasites; very often cells contain parasites in different phases of development (Pl. I, fig. 3). In the case of *T. cruzi*, for instance, the transformation of the leishmania into the flagellated form takes place only when the host cell is completely filled. It is only then that the cell begins to

degenerate, ruptures and frees the trypanosomes. *Toxoplasma* also fills the host cells completely, which show degeneration before they eventually rupture. We are not yet certain that the rupture of a single schizont and the departure of the parasites, in *P. gallinaceum*, always means the death of the host cell. Observations on this problem still continue in our laboratory, and an exact description of the various phases of the life cycle of *P. gallinaceum* and its relation to the host cell must be postponed.

Since the initial forms are difficult to recognize *in vivo*, their direct observation was always followed by fixation and staining with Giemsa, in order to avoid mistakes and to be certain that the observed inclusion was really a parasite and not one of the common cell inclusions that occur normally in tissue cultures. When the rupture of a schizont took place in a microscopic field which was easy to identify later, the culture was fixed after 12, 24 and 48 hr. As we stated in a previous paper, the cycle of *P. gallinaceum* in tissue cultures seems to be slower than has been observed by Huff & Coulston (1944) in animals, since 12 hr. after the rupture of a cell, the phagocytized parasites are only small, round bodies, which show increased size in preparations which are fixed after 24 hr., and division of the nucleochromatin in the 48 hr. preparations. These observations must be repeated because it is possible that the parasites were not phagocytized immediately after their liberation. However, since we know that they degenerate rapidly outside the cell, this source of error is limited to the very first hours.

PASSAGE FROM THE EXO- INTO THE ENDOERYTHROCYTIC FORM *IN VITRO*

A culture of *P. gallinaceum* maintained for a long period *in vitro* gives, when inoculated into a chick, an exoerythrocytic infection, which later passes to an infection of the blood. An attempt was made to obtain the same passage of the exo- into the endoerythrocytic forms *in vitro*. A heavily infected tissue was placed in a Carrel flask and covered with a drop of normal chicken blood which was allowed to clot on top of the tissue culture. After 48 hr. incubation, the cultures were washed and covered with heparinized chick plasma, and were left for 2–3 hr. in the thermostat. The heparinized plasma was then removed. This technique was advised by Fischer and Parker for a slow growth of tissue cultures and proved useful in the cultivation, of the infected brain tissue in our previous experiments. After 48 hr. of cultivation and at daily intervals for 5 days, samples of the liquid above the culture were removed with a capillary pipette and were examined in a fixed smear stained with Giemsa. Despite the fact that many unpigmented free forms were found in almost all the preparations (Pl. I, fig. 4), and that the red blood cells were found in good condition, not one of the preparations which were examined showed pigmented parasites.

MAINTENANCE OF *PLASMODIUM GALLINACEUM* IN TISSUE CULTURES AT ROOM TEMPERATURE

In previous experiments we have demonstrated that it is possible to keep intracellular parasites in tissue cultures which were maintained at room temperature without transfer or any other treatment after a short incubation of 24 hr. Under

these conditions the tissue cells survive for about 3 weeks, and with them, the parasites which they contain in the cytoplasm. Since this fact is of great practical value for the routine work of the laboratory, we repeated these experiments with *P. gallinaceum*.

Infected cultures, in hanging drops, were incubated for 24 hr. and thereafter stored in the dark at room temperature. After a period varying from 12 to 20 days at room temperature, the cultures, in contact with fresh tissue, were transferred to roller tubes. After about 8 days of cultivation in roller tubes, the cultures were transferred again to hanging drops and direct observation in the microscope showed parasites in those cultures which have been maintained for 23 days to date, at room temperature.

SUMMARY

P. gallinaceum was cultivated in tissue cultures for 1 year. The cultures showed the same high-grade infection as has been observed previously with *Toxoplasma* and *Trypanosoma cruzi*, and made possible new observations upon the living parasite.

It has not been possible to obtain *in vitro* the passage from the exo- into the endoerythrocytic form.

The parasites can survive in tissue cultures, maintained at room temperature, for 23 days.

Observations concerning the periodicity and the phases of the cycle in tissue cultures are being investigated.

EXPLANATION OF PLATE I

(In all cases the photographs were made with Leica and Bausch-Lomb achromatic microscope, objective 90×, ocular 10×.)

Fig. 1. Cult. 6188M. Infected brain tissue. Large quantity of parasites. Bouin-Giemsa.
Fig. 2. Cult. 9260H. Infected brain tissue. Cell containing segmenters. Bouin-Giemsa.
Fig. 3. Cult. 6539M. Infected brain tissue. Cell containing schizonts in different stages of development. Bouin-Giemsa.
Fig. 4. Cult. 6079M. Infected brain tissue, together with normal blood. Unpigmented schizonts in the liquid removed from the culture. Metanol-Giemsa.

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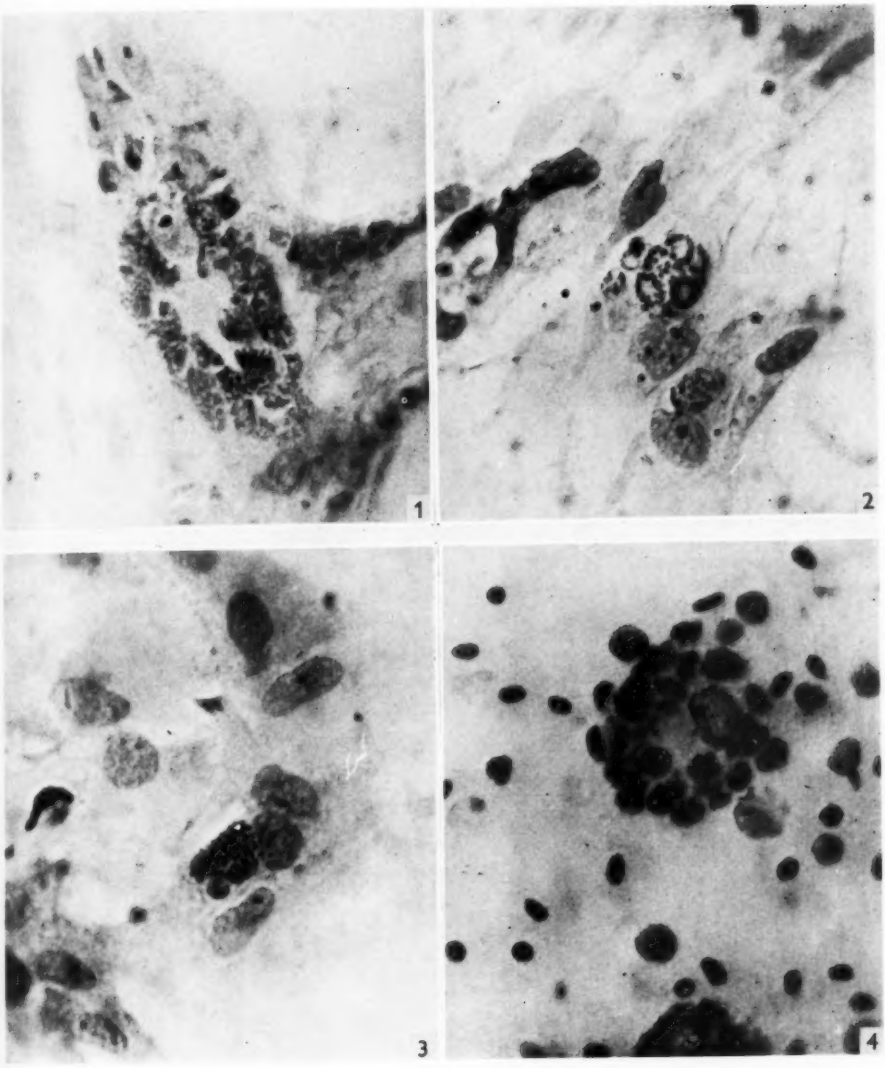

(*Facing p.* 4)

ANATOMY AND AFFINITY OF THE ELEPHANT LOUSE *HAEMATOMYZUS ELEPHANTIS* PIAGET (INSECTA: RHYNCOPHTHIRAPTERA)

By D. MUKERJI, M.Sc. and P. SEN-SARMA,* M.Sc.

Entomological Laboratory, Department of Zoology, Calcutta University

(With 40 Figures in the Text)

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INTRODUCTION

The elephant louse, *Haematomyzus elephantis* Piaget, had attracted the attention of entomologists since 1869, when it was recorded from the Indian elephant from Ceylon. But no clear knowledge of it was available until Ferris (1931) gave a review of previous work on it and showed the mandibulate nature of its mouthparts. He separated it from the order Anoplura, in which this insect had been classified by previous workers, and put it as a suborder of Mallophaga. Weber (1938, 1939*a*, *b*) described its bionomics with a brief reference to its internal anatomy. Snodgrass (1944) confirmed that it is a mandibulate type. Webb (1946), who studied its spiracles, expressed the view that the elephant louse *Haematomyzus*, and the pig louse, *Haematopinus*, were closely related genera. The object of this paper is to give a more complete account of its morphology and to discuss both the affinity and the possible origin of this insect.

MATERIAL AND METHODS

Live specimens of *Haematomyzus elephantis* Piag. were collected from the Indian elephants that passed through the hands of the animal dealers at Calcutta for export. These parasitic insects infest the skin of the host, hiding within the integumentary folds in the region of the groin, the armpit, the root of the tail, and the ear. But they are hardly seen in well-groomed hosts. The host specificity is indicated by the fact that this louse does not survive more than 3 hrs. when it is taken out of the elephant and is kept in a glass vial or when it is transferred to the body of a guinea-pig, or any other mammalian host. The female insects are found in

* Now at the Forest Research Institute, Dehra Dun, India.

larger number than the males, as reported by Weber (1939). The specimens were treated with 10% KOH and mounted in Berlese's medium. For study of internal anatomy, fresh specimens were dissected in normal saline solution (0·75%), to which a few drops of weak chromic acid were added. Incision on the abdomen was made along the ventro-lateral edge and a mid-ventral incision was made in the region of the head and thorax. The integument was carefully removed to expose the soft parts which were fixed in Carnoy and stained in Grenacher's Borax carmine. For serial sectioning, Carnoy-Lebrun solution was found to be the best fixative. Softening of chitin was done by treating the fixed specimens for a fortnight in a solution of 7% nitric acid in 70% alcohol. Double embedding in celloidin (celloidin dissolved in ether and absolute alcohol in the proportion of 1:1) and paraffin, was used. For sticking the sections to the slides Mukerji's (1937) method was adopted. Staining was done by Mallory's triple stain, and also by iron-alum haematoxylin, Orange G being used as a counter-stain.

HEAD CAPSULE

The head, when it is viewed from the top, is triangular in outline (Fig. 1). Its dorsal and ventral surfaces are arched except at the anterior end beyond the level of the eyes, where the ventral surface becomes flat. The dorsal surface is strongly chitinized and dark brown in colour; the ventral surface is soft, membranous and non-pigmented, except at the posterior region which is lightly pigmented. The dorsal surface, on the level of the eyes, bears a sensory seta. Anteriorly in the middle region, the head is prolonged into a snout called by Ferris (1931) the rostrum, which is a uniformly cylindrical tube. The apex of the rostrum is remarkable for its dark pigmentation; it bears the mouthparts. The antero-lateral angles of the head are produced at the basal region of the rostrum, into a pair of thick conical processes.

At the ventral surface of the head, there are rows of denticles, the apices of which are rounded and directed backwards, while their bases look like scales with overlapping edges. They were described by Ferris (1931) as squamate-reticulate in form. The pattern is better understood in longitudinal sections of the head (Fig. 21). The fine chitinous serrations, arranged in linear rows on the ventral surface of the integument, are sections of the overlapping edges.

At the posterior end of the head, the occipital region is broad. Two chitinous knobs project from the ventral surface of the occipital region (Figs. 11, 21). Possibly, they prop the head up when the louse keeps its body pressed against the integumentary folds of the elephant.

The posterior extremity of the head capsule is produced medially backward in the form of a triangular lamina which passes through the thorax posteriorly as far as the level of the coxae of the second pair of legs (Fig. 2, 21). This lamina is chitinous. Its origin from the mid-dorsal region of the head is not marked by any sutural line. The lamina lies free within the thorax. Bands of transverse muscles pass beneath the ventral surface of the lamina and tie together the two lateral edges of the lamina (Fig. 14). By contraction of these muscles, the lamina can either bend in the middle or be rolled into an imperfect tube. Ferris (1931) describes this lamina

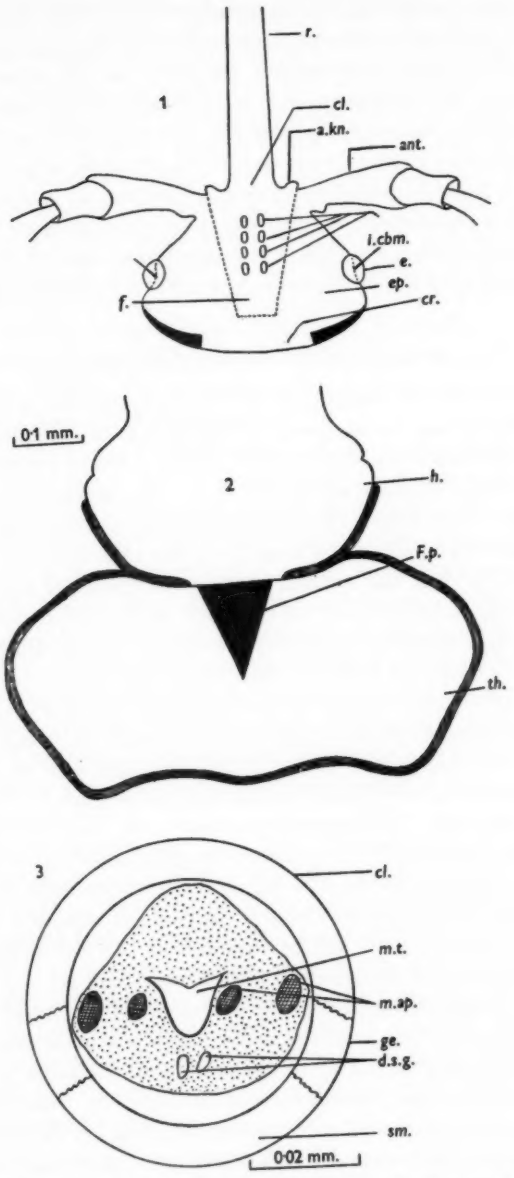

Fig. 1. Schematic diagram of the head capsule. *r.* rostrum; *cl.* clypeus; *a.kn.* anterior knob; *ant.* antenna; *i.cbm.* impression of dorsal cibarial muscles; *e.* eye; *ep.* epicranium; *f.* frons; *cr.* cranium.

Fig. 2. Head and thorax showing the Ferris plate (*F.p.*); *h.* head; *th.* thorax.

Fig. 3. Transverse section of the rostrum through the submentum. *cl.* clypeus; *m.ap.* mandibular apodemes (extensor and flexor ligament); *m.t.* mouth-tube; *ge.* gena; *d.s.g.* ducts of salivary gland; *sm.* submentum.

as a median hollow apophysis, which opens at the surface by a narrow slit. We have, however, found that it is not hollow and that it does not open to the exterior. The homology of this plate is uncertain, because a similar structure is not known in any other insect. But, because no cervical region is distinguishable in this species, it is possible that the lamina represents the dorsal cervical sclerite that has been pushed deep inside the thorax to act as a lever, perhaps, as a spring, of the head, when the head is moved up and down during feeding. It is equally possible that, while it is within the thorax, this sclerite acts like a cushion which absorbs the shock of compression when the thorax is pressed hard against the hide of the host. The plate is named here the *Ferris plate*, as a tribute to Ferris who called attention to it.

HOMOLOGY OF THE HEAD CAPSULE

The head capsule shows no suture and therefore appears like a single piece, but its constituent elements can be analysed by reference to the position and origin of the musculature, in accordance with the principle laid down by Snodgrass (1939, 1947). In delimiting the constituent areas, we have also taken into consideration the relative positions of the brain, the frontal ganglion, the eye and the antenna.

The dorsal cibarial muscles originate from the middle of the roof of the head capsule (Fig. 1, 21). For this reason, the part of the head capsule to which the cibarial muscles are attached may be regarded as the clypeus (Fig. 1). The latter is thus a large sclerite forming the roof of the entire rostrum, although Ferris (1931) considered that the clypeus is either absent or reduced.

The next area of the head capsule, situated behind the clypeus, and lying between the origin of the precerebral pharyngeal muscles and the frontal ganglion, represents the frons, which is, however, very small and narrow (Figs. 1, 21). We do not agree with Ferris that the entire anterior portion of the head, including the rostrum, corresponds to the frons. The epicranium in our view lies just behind the frons; it extends forwards, however, by the side of the clypeus and frons so that it reaches the inner margin of the antenna. The triangular apotome said to be splitting open in continuity with the dorsal wall of the rostrum, as reported by Weber (1939), from his study of the exuviae, can be regarded as the fronto-clypeus, so that its posterior margin should correspond to the epicranial suture.

The cranium proper forms the posterior region of the head capsule and lies behind the epicranium, from which it is not sharply demarcated (Fig. 1).

The sides of the head just below the eyes represent the parietal region, which is, however, very narrow and inconspicuous, because the head is dorso-ventrally flattened. The gena forms the sides of the rostrum (Fig. 3). It is fused on the dorsal edge with the clypeus and ventrally it is fused with the submentum and mentum. Notwithstanding fusion, the gula is well developed and covers a considerable portion of the ventral region of the head, extending forward as far as the base of the rostrum. The submentum starts from where the gula ends; and the mentum is in front of the submentum. No line of demarcation between the mentum and submentum can be detected.

ANTENNA

The antenna is five-jointed in both the males and the females (Fig. 4). It is lighter in colour than the head and subequal in length to the rostrum. The scape is the largest joint. In this character, the elephant louse differs markedly from both the Anoplura and the Mallophaga in both of which, the first joint is nearly equal in length to the next. The scape is narrow at the proximal end and gradually broadens out at the distal end (Fig. 6). The pedicel is twice as long as it is broad and is nearly as long as the third and the fourth joints put together. Unlike other joints, the pedicel is uniform in width. The third and the fourth joints are subequal in length. The last joint is the smallest. It is subcylindrical and carries at its apex the sensory spines, which are more or less similar to those in the Mallophaga. It may be noted that the sensory pits found laterally on the fourth and the fifth joints of the antennae of the Anoplura are lacking in this species, as Ferris (1931) pointed out. Likewise, the lateral pits are absent in Mallophaga.

The antennal muscles pass from the head capsule to the scape, but do not go beyond that. In this respect *H. elephantis* resembles *Haematopinus tuberculatus*, described by Qadri (1948), *Haematopinus suis*, described by Florence (1921), and *Pediculus humanus*, described by Keilin & Nuttall (1930). The muscles originating from the head capsule and supplying the base of the antenna are six in number, three being present on each side. The components are as follows: a short oblique muscle runs through the outer border of the head, its origin being ahead of the eye (Fig. 5). Two parallel muscles originate from the roof of the head at one-third the distance from the posterior extremity of the head. These run a straight course and are applied against the dorsal side of the scape, near the junction of the latter with the head (Fig. 5). The point of origin of this straight pair of muscles lies in front of the level of the supra-oesophageal ganglion, but posterior to the elevator muscles of the cibarial pump.

In the scape, the muscles are six in number, of which two are on the dorsal, two on the ventral and one on each lateral side (Fig. 6). The dorsal pair is medial in position. They arise from the junction of the scape with the head and end on the junction of the pedicel with the scape. The dorsal pair elevates the antenna, the antenna being raised up as these muscles contract. The ventral pair depresses the antenna. The origin, insertion and course of this pair are the same as those of the dorsal pair, except that the muscles are ventral in position. The lateral muscle which runs on the anterior border of the antenna, acts by flicking the antenna forward, while the muscle band running on the posterior border of the antenna draws the antenna backward.

MOUTHPARTS

The mouthparts are crowded at the tip of the rostrum. The latter, anteriorly on the dorsal side, terminates in a small flat plate. This we term the labrum, because of its position in front of the clypeus, from which it is separated by a faint suture (Fig. 7). The apex of the labrum is incised in the middle, giving rise to the right and the left apical lobes. Each lobe is small in size and is furnished with a sensory seta, which is conspicuously long and is directed forward and upward. Posterior to the

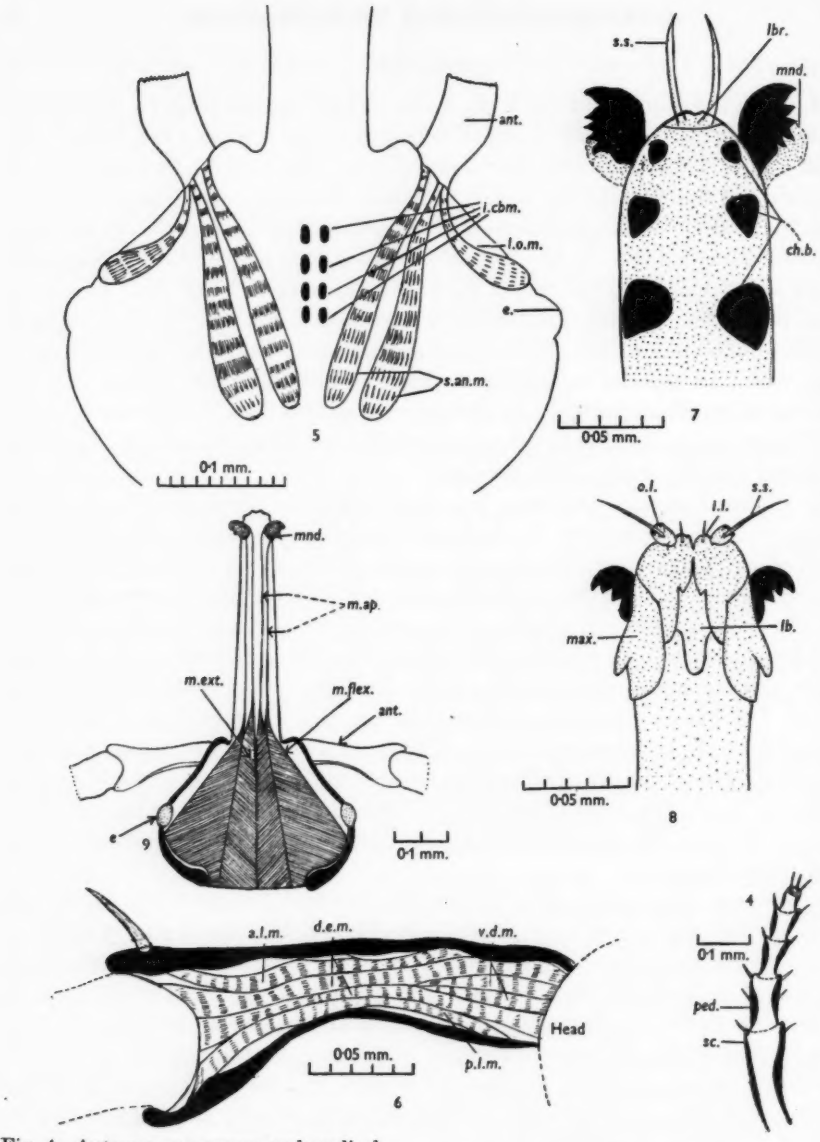

Fig. 4. Antenna. *sc.* scape; *ped.* pedicel.

Fig. 5. Diagrammatic representation of the antennal muscles within the head. *ant.* antenna (scape shown); *i.cbm.* impression of dorsal cibarial muscles; *l.o.m.* longitudinal oblique muscle; *e.* eye; *s.an.m.* straight antennal muscles.

Fig. 6. Scape of antenna to show the muscles within. *a.l.m.* antero-lateral muscle; *d.e.m.* dorsal elevator muscles; *v.d.m.* ventral depressor muscles; *p.l.m.* postero-lateral muscle.

Fig. 7. Dorsal view of the tip of the rostrum. *s.s.* sensory seta; *lbr.* labrum; *mnd.* mandible; *ch.b.* chitinous bosses.

Fig. 8. Ventral view of the tip of the rostrum. *i.l.* inner lobe; *o.l.* outer lobe; *s.s.* sensory seta; *max.* maxilla; *lb.* labium.

Fig. 9. Head showing mandibular muscles. *mnd.* mandible; *m.ap.* mandibular apodemes (extensor & flexor ligament); *m.ext.* extensor muscles; *m. flex.* flexor muscles; *ant.* antenna; *e.* eye.

labrum, three pairs of strongly chitinous bosses follow serially along the length of the rostrum. These are anchylosed to the dorsal surface of the rostrum. They lie equidistant from one another (Fig. 7). The first pair of bosses is the smallest of the series. They are placed on either side of the median line of the rostrum near the anterior level of the projecting ends of the mandibles. The second pair lies behind the first pair and is situated posterior to the level of the projecting ends of the mandibles (Fig. 7). The second pair is intermediate in size between the first and the third pair; they are triangular in outline. The third pair is the largest in size and is somewhat cordate in shape. These chitinous bosses fall out if the specimens are treated with KOH solution and leave behind chitinous pits. The significance of these bosses is not clear; it is possible, however, that they act as odoriferous sense plates or are tactile organs, although no pits or pores were observed on their outer covering, and nerve endings on these could not be definitely traced.

The ventral surface of the rostrum at the anterior end is provided with a pair of lateral, and a median unpaired, sclerotic plate (Fig. 8). Each lateral plate bears at the apex sensory setae. Ferris (1931) states that these plates represent the maxillae. The median plate, because it is flanked on either side by the maxillary plate, evidently represents the labium. The labium is thus not so ill defined as Ferris (1931) thought it to be.

Each maxilla can be distinguished, by the presence of a faint suture, into an anterior apical portion and a posterior basal portion (Fig. 8). The apical portion is subdivisible into an inner and an outer lobe. The inner lobe is globular with a wavy outline. It is furnished with a small sensory seta which points downwards. The outer lobe is more or less cylindrical. It gives out from its middle a long seta that projects forwards and outwards. The basal portion of the maxilla, posteriorly, is cut into an inner and an outer wedge-shaped lamina. Ferris (1931) suggests that the two maxillae are movable by means of two pairs of fine apodemes. But we have not found these apodemes, either in our dissections or serial sections. We consider that the two maxillae are degenerate structures in the sense that they have lost muscles, nerves and other associated structures. In our opinion, the maxillae are not movable; they are firmly fixed to the mentum having lost their original function.

The rostrum, towards the anterior end, contains on either side a small aperture for the passage of the terminal end of the mandible. The blade of the mandible is semi-circular in outline. The mandible possesses ball and socket joints. A condylar process arises dorsally from the inner wall of the apical plate of the rostrum and this fits into a socket present on the dorsal aspect of the mandible, as stated by Ferris (1931). On the ventral side, the mandible has a condyle which fits into a socket placed on the ventral aspect of the rostrum. Thus the elephant louse is, as Ferris (1931) pointed out, curious in having the mandibular condyle articulating with the ventral wall of the head, instead of with the lateral wall. Each mandible is provided with an extensor and a flexor ligament. The extensor ligament is attached to the dorsal surface of the mandible, which contains the mandibular socket for reception of the condylar process of the dorsal plate of the rostrum. The flexor ligament, on the other hand, is attached close to the mandibular condyle

(Fig. 9). Both the extensor and the flexor ligaments run parallel to each other within the rostrum and pass inside the head for attachment to the extensor and flexor muscles respectively. The extensor muscles lie on either side of the pharynx. These originate from the postero-dorsal wall of the head capsule and spread over a wide area (Fig. 9). The flexor muscles lie on either side of the extensor muscles. They also expand posteriorly, originating from the dorsal wall of the head capsule near the postero-lateral angles (Fig. 9).

The mandible has its outer surface cut into numerous serrated teeth, which are crowded together. The anterior tooth is claw-like. The teeth bearing arc of the mandible projects laterally to pass to the exterior. It is curious that these teeth are turned outwards, that is to say, away from the main axis of the body, so that the teeth of one side do not oppose those of the other. The teeth can, therefore, neither bite the food nor pass it into the mouth. This curious feature may be explained by assuming that each mandible rotated through an angle of 180° around the main axis. This resulted in change of direction of the toothed surface from inward to outward, the change leading to loss of biting function, but tending to help attachment to the host. Because no other ecto-parasitic insects, not even the biting lice, show the peculiar feature of un-opposable teeth on the mandible, we presume that the elephant louse arose from an ancestor distinct both from the Mallophaga and the Anoplura. Although the disposition of claw-like teeth, suggests fixation to the skin of the host, it is not improbable that the teeth cut or abrade the skin, because a large quantity of blood was found in the gut of this insect. We believe that blood oozing from the scratched surface of the host is sucked in by applying the mouth to the site of the wound, which could be, either on the right, or on the left of the mouth aperture, according as it was caused by the teeth of the right or the left mandible. The wound is caused when the mandibles are thrust out through the lateral apertures of the rostrum, which are, it should be noted, quite separate from the median mouth opening situated at the extremity of the rostrum.

THORAX

The thorax of the elephant louse is dark brown in colour. It is a compact and strongly chitinized structure, all sign of segmentation and suture being effaced. Viewed from the top, it is rectangular in shape, but the posterior angles are drawn out and somewhat pointed. On the dorsal side a pale anterior area is distinguishable (Fig. 10). As Ferris (1931) stated, this corresponds to the pronotum because the first pair of legs are attached on the ventro-lateral surface of this area. This segment does not bear any spiracle, although Webb (1946) wrongly locates a pair on it. Unlike the pronotum, the meso- and the metanota are not well defined. These are completely fused, no trace of sutural line remaining.

From the postero-dorsal wall of the metathoracic region two chitinous apophyses arise and these project deep within the abdomen.

A small triangular chitinous plate is seen near the bases of the coxae of each of the second and the third pair of legs (Fig. 11). Ferris (1931) considers that these plates

are remnants of the degenerated sternal plates of the meso- and the meta-thorax respectively. It may be mentioned that no kind of a plate or remnant of it is found at the base of the prothoracic coxa. The ventral surface of the thorax evidently had been formed by extension and fusion of the pleurites, because the contribution to its formation by the sternites, which either exist in an extremely reduced form, or are evanescent, as they are in the prothoracic region, could be only negligible.

A strongly chitinous pair of rods, issuing out from the median ventral lobe of the occipital foramen of the head, passes within the thorax. This pair of rods runs posteriorly as far as the level of the insertion plane of the coxae of the second pair of legs. Then each bends back to pass forwards to end on the coxa of the first pair of legs corresponding to that side (Fig. 11). According to Ferris (1931), the rods represent the ventral apophyses, because in the Anoplura, an equivalent structure arises from the thoracic sternum near the anterior coxae. We, however, believe that this framework represents the pleuro-sternal complex or propectus of Snodgrass, which serves as a suspensorium for the first pair of legs as well as a support for the head, because a corresponding separate plate in the prothorax is found wanting. Moreover, these rods are distinct from the metathoracic apophyses that run within the abdomen.

LEGS

The legs of the elephant louse are peculiar in that each has a slender claw which, unlike that of the sucking lice, is devoid of the thumb, and is incapable of grasping the hair of the host. The legs of *H. elephantis* do not show the sexual dimorphism reported by Keilin & Nuttall (1930) in the sucking lice.

The coxa is short and globular. The coxae of the first pair of legs are smaller than those of the second and the third pair of legs. The trochanter is small and its lateral margins are slightly notched.

The femur is well developed. As compared with the short femur of the Anoplura, it is slender and long. Its upper margin is slightly convex, but its lower surface is straight and is furnished with stout pointed spines (Fig. 12). The femur of the first leg is smaller than the femora of the second and the third legs which are of equal size. Both the tibia and the tarsus are long and slender. Towards the distal end of the tarsus there is a faint dividing line, which suggests the existence of a pre-tarsus; this line escaped the notice of Ferris (1931). Because the integument across the dividing line is membranous, this seems to be a junction (Fig. 12). Two chitinous plates exist, as they do in Anoplura, at the distal end of the pre-tarsus (Figs. 12, 13). One of them is elongate and is serrated. We call this a *serrate plate*. It is situated, if the leg is held parallel to the long axis of the body, at the outer border of the pre-tarsus. The serrate plate extends forwards and is firmly connected with the claw. A fine apodeme arising anteriorly from the femoral muscles connects the serrate plate to the femoral muscles. The other plate, situated at the inner border of the pre-tarsus, is elliptical and non-serrated. The pre-tarsus ends in a single claw, which is finely serrated on its ventral side. Near the junction of the claw with the pre-tarsus, a long curved seta arises. Ferris (1931) assumes this to be a secondary claw. We believe it to be a sensory seta or a tenet hair.

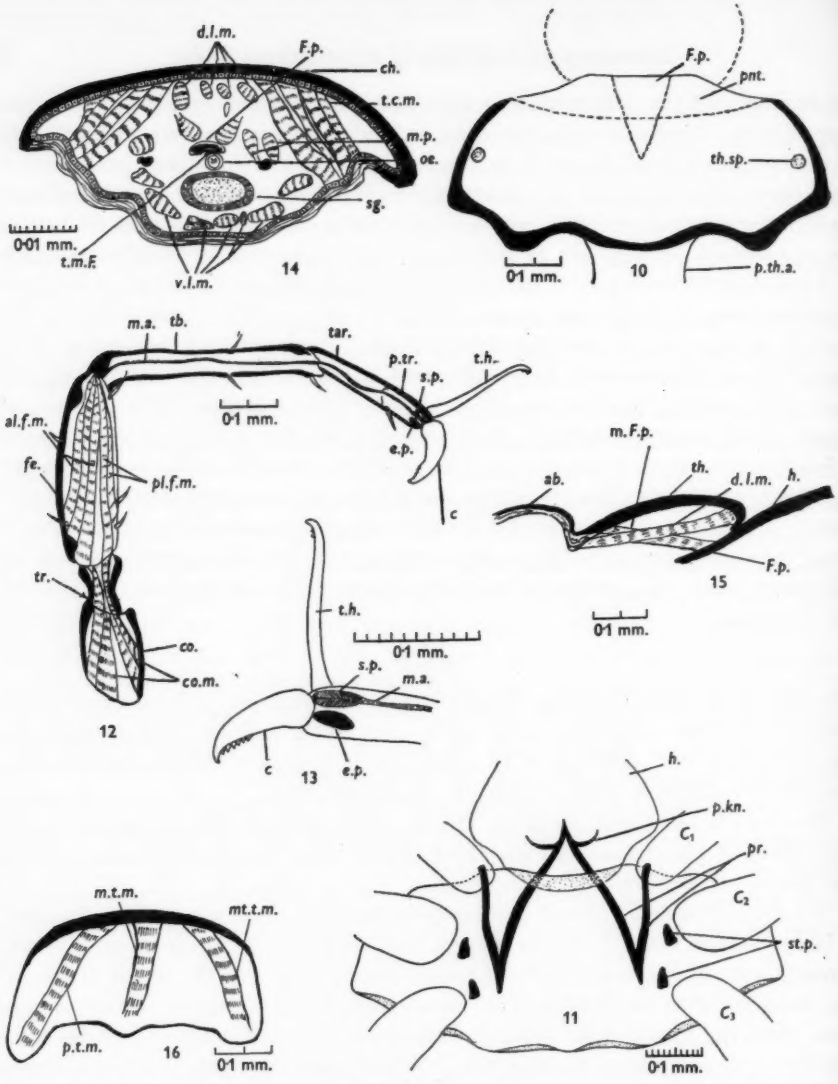

Fig. 10. Dorsal view of the thorax. *F.p.* Ferris plate; *pnt.* pronotum; *th.sp.* thoracic spiracle; *p.th.a.* posterior thoracic apophysis.

Fig. 11. Ventral view of the head and thorax. *h.* head (posterior part); *p.kn.* posterior knob of head; *pr.* propectus; C_1, C_2, C_3, 1st, 2nd and 3rd coxa respectively; *st.p.* sternal plates.

Fig. 12. 3rd leg and its muscles. *co.* coxa; *co.m.*, coxal muscles; *tr.* trochanter; *fe.* femur; *al.f.m.* anterolateral femoral muscles; *pl.f.m.* postero-lateral femoral muscles; *m.a.* muscular apodeme; *tb*, tibia, *tar.* tarsus; *p.tr.* pre-tarsus; *s.p.* serrate plate; *e.p.* elliptical plate; *c.* claw; *t.h.* tenet hair.

Fig. 13. Pre-tarsus and claw shown magnified. *t.h.* tenet hair; *m.a.* muscular apodeme; *s.p.* serrate plate; *e.p.* elliptical plate; *c.* claw.

Fig. 14. Transverse section of thorax. *d.l.m.* dorsal longitudinal muscles; *v.l.m.* ventral longitudinal muscles; *F.p.* Ferris plate; *t.m.F.*, transverse muscle to Ferris plate; *ch.* chitin; *t.c.m.* tergo-coxal muscles; *m.p.* muscles to propectus; *oe.* oesophagus; *sg.* suboesophageal ganglion.

Fig. 15. Longitudinal section of thorax. *F.p.* Ferris plate; *h.* head; *th.* thorax; *ab.* abdomen; *d.l.m.* dorsal longitudinal muscle; *m.F.p.* longitudinal muscle to Ferris plate.

Fig. 16. Longitudinal section of thorax showing origin of tergo-coxal muscles (diagrammatic). *p.t.m.* protergo-coxal muscle; *m.t.m.* mesotergo-coxal muscle; *mt.t.m.* metatergo-coxal muscle.

MUSCULATURE OF THE THORAX AND THE LEGS

In the thorax, there are four dorso-longitudinal muscles, and five ventro-longitudinal muscles. The dorso-longitudinal muscles connect the inflected postero-dorsal wall of the meta-thorax with the anterior region of the prothorax (Fig. 15). The ventro-longitudinal muscles pass from the inflected ventral wall of the meta-thorax to the ventral wall of the prothorax at the region where the head and the thorax meet. It is worth noting that the corresponding ventro-longitudinal muscles were shown by Qadri (1948) as absent from *Haematopinus tuberculatus*.

Another pair of muscles of the dorsal group arises from the postero-dorsal wall of the meta-thorax slightly anterior to the origin of the former. These run obliquely downwards and forwards, passing over the dorsal surface of the Ferris plate, which arises from the posterior wall of the head capsule and runs within the thorax (Fig. 15). A pair of thoraco-abdominal muscles, one on either side of the median line of the body, connects the anterior edge of the first abdominal segment with the posterior edge of the thorax. They follow an oblique course from the dorsal to the ventral surface.

There are three groups of tergo-coxal muscles, which correspond to the three divisions of the thorax; these supply the three pairs of legs. Each group of tergo-coxal muscles consists of two pairs lying laterally on either side of the dorso-longitudinal muscles (Fig. 14). The protergo-coxal muscles, supplying the first pair of legs, arise from the thoracic tergum, slightly ahead of the mesotergo-coxal muscles. They run forwards and downwards and are inserted on to the anterior and the posterior margins of the bases of the coxae of the first pair of legs. The mesotergo-coxal muscles originate from near the middle of the tergum of the thorax. They run downwards and are inserted on the coxae of the second pair of legs at the anterior and the posterior basal margins. The metatergo-coxal muscles arise from the tergum just behind the origin of the mesotergo-coxal muscles; they are inserted on the coxae of the third pair of legs (Fig. 16).

Each coxa contains four muscles of which two are antero-lateral and two are postero-lateral in position, if the leg is held parallel to the long axis of the body. They arise from the anterior and posterior margins of the base of the coxa, converge backwards and are inserted on the lower margin of the trochanter (Fig. 12). The trochanter is supplied with only two muscles, which arise from the base of the trochanter and diverge to terminate on the lower basal margin of the femur.

There are five bands of muscles within the femur, of which three are antero-lateral, and two are postero-lateral, if the leg is held parallel to the long axis of the body. The three antero-lateral muscles arise from the antero-lateral margin of the base of the femur, run forwards and are inserted on the anterior wall of the base of the tibia. The two postero-lateral muscles of the femur arise from the postero-lateral basal margin of the femur and, running forwards, are also inserted on the anterior wall of the tibia, close to the insertion of the antero-lateral muscles (Fig. 12). The muscle-elements of the tibia, tarsus and pre-tarsus, present in the Anoplura, are absent from *H. elephantis*. Instead, a fine chitinous apodeme, connected at one end with the femoral muscles at the region where these muscles are

inserted on the tibia, runs medially within the tibia and tarsus. Within the pre-tarsus, the chitinous apodeme is connected with the serrate plate, which lies at the antero-lateral tip of the pre-tarsus.

ABDOMEN

The abdomen is oval in shape (Figs. 17, 18). The dorsal surface is dark brown, while the ventral surface is yellowish. The dorsum of the abdomen is very thick and it is strongly chitinized; but the ventral surface is membranous and soft. The ventral surface is plastered with overlapping denticular scales similar to those on the head but less elevated. These were described by Ferris (1931) as squamate reticulate. Because the abdomen of the female differs from that of the male, the two are described separately.

Female

The abdomen of the female consists of eight visible segments, which are sharply delimited at the lateral margins by inter-segmental constrictions (Fig. 17).

The abdominal tergite in a segment usually consists of a median and two lateral plates, which lie one on either side. The median plate of the tergite is separated from the lateral plates by a membranous area, which belongs, perhaps, to the intersegmental region that is displaced. The lateral tergites, because of their position, cannot be mistaken for the pleurites, which are distinct and are dorso-lateral in position. The position of the spiracle clearly delimits the lateral tergite from the pleurite. On the first segment, the median tergite is represented by a narrow, rectangular plate. The lateral tergites and the spiracles are absent here. The second segment, curiously enough, bears two mid-dorsal tergites, one behind the other, and on either side a single lateral tergite and a spiracle (Fig. 17). The presence of double mid-dorsal tergites, although it suggests that the second segment is a double segment can be explained as having arisen by division of the mid-dorsal tergite into two, because the corresponding lateral tergites and spiracles maintain the number and position characteristic of a single segment. The third, fourth and fifth segments are more or less similar to one another, each bearing a single mid-dorsal tergite, a pair of lateral tergites, and a pair of spiracles (Fig. 17). The sixth segment, like the second, bears two mid-dorsal tergites, one behind the other, a pair of lateral tergites and a pair of spiracles. In the seventh segment, the median tergite is represented by a large mid dorsal plate, which covers the entire dorsal region of this segment. The lateral tergites are absent or are fused with the mid-dorsal tergite and lose their identity. The genital opening is on the ventral side of this segment. The pleurites are small and inconspicuous (Fig. 19). The last (eighth) segment is remarkable because both the mid-dorsal and lateral tergites and the pleurites and spiracles also are absent. This segment is greatly reduced in size and is membranous. Because the anal aperture is situated at the terminal end of this segment, we call this the *anal segment.*

Male

The abdomen of the male is comparatively slender (Fig. 18). The mid-dorsal tergite of the first segment is, unlike that of the female, broad. The lateral tergite is absent as it is from the female. Of the two mid-dorsal tergal plates, in the second

segment, the first remains free and the second becomes fused laterally with the lateral tergites of the same segment, and ventrally with the mid-dorsal tergite of the third segment. In the subsequent segments, from the third to the sixth, the mid-dorsal tergites and the lateral tergites, unlike the corresponding tergites of the female, are fused together, but the sutural lines remain distinct (Fig. 18). The genital plate mentioned by Ferris (1931) is absent. The disposition of the spiracles is the same as it is in the female.

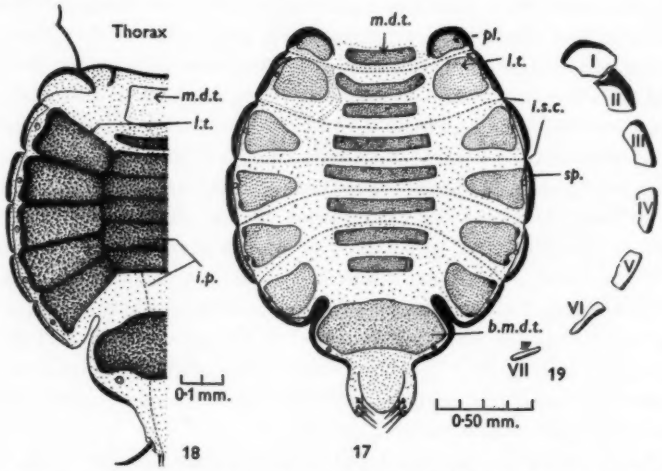

Fig. 17. Abdomen of the female (dorsal view). *m.d.t.* mid-dorsal tergite; *pl.* pleurite; *l.t.* lateral tergite; *i.s.c.* inter-segmental constrictions; *sp.* spiracle; *b.m.d.t.* broad mid-dorsal tergite.

Fig. 18. Abdomen of the male (dorsal view). *m.d.t.* mid-dorsal tergite; *l.t.* lateral tergite; *i.p.* impression of male genitalia.

Fig. 19. Pleurites shown magnified. Pleurites on the lateral side are shown black, while those on the dorsal side shown white.

ALIMENTARY CANAL

The alimentary canal is more or less a straight tube as in sucking lice. The mouth opening is a narrow slit at the extreme anterior end of the rostrum. This leads into a narrow tube called the *mouth-tube*, which traverses the entire length of the rostrum and the anterior portion of the head, to open into the *cibarial pump* (Fig. 20). The chitinous lining of the mouth-tube is thin on the dorsal side, but the rest of it is thick. Epithelial cells and muscle-elements are absent from its wall. The mouth-tube can be homologized with the hypopharynx by assuming that the latter has been converted into a suctorial tube by curving and fusion of its edges. The blood meal is sucked into the mouth-tube by the cibarial pump, which is situated within the head towards the anterior end. The pump or the *cibarium*, as Snodgrass (1944) terms it, is a curved tube having a concavity above and a convexity below. The cibarium is lined by a chitinous intima, which is moderately thick, but no epithelial layer is discernible in it. The cibarium possesses two groups of dilator muscles: the dorsal and the ventral (Fig. 21). The dorsal dilators of the cibarium are four in

number. They arise from the clypeus and converge down to be inserted on the mid-dorsal wall of the cibarium. The ventral dilator muscles, on the other hand, arise from the middle of the floor of the head and spread out gradually to be attached on the ventral wall of the cibarium (Fig. 21). By synchronous contraction of the dorsal and the ventral dilators, the cavity of the cibarium is widened and the blood of the host flows into the vacuum thus created. On relaxation of these muscles, the cibarium contracts by virtue of the elasticity of its wall. The sharp curvature of the pump prevents regurgitation and the ingested blood is forced down into the pharynx.

The *pharynx* is a short tube occupying the posterior third of the head. The pharynx soon after its origin, dips down. In transverse section, it has the shape of a cross (Fig. 22). We did not find any cellular lining in the wall of the pharynx. The pharynx is provided with extrinsic and intrinsic muscles. The extrinsic muscles include the dorsal and the ventral pharyngeal dilators (Fig. 22). The dorsal pharyngeal dilators originate from the dorsal wall of the cranium at a little distance behind the origin of dorsal cibarial dilator muscles. These muscles are inserted on the dorsal wall of the pharynx, just posterior to the level of the frontal ganglion (Fig. 21). The ventral pharyngeal dilators, on the other hand, arise from the floor of the head, behind the origin of the ventral cibarial dilators. They are inserted on the ventral wall of the pharynx, posterior to the level of insertion of the dorsal pharyngeal dilators (Fig. 21). The intrinsic muscles of the pharynx are the strong band of circular muscles around the pharynx, and the longitudinal muscle fibres laid outside and inside the circular muscles.

The pharynx leads into the *oesophagus*, which is a thin-walled tube. It extends from the posterior portion of the head to the thorax (Fig. 20). The intima lining the oesophagus is thinner than that lining the pharynx; its epithelial cells are narrow and sparse in number. The disposition of its muscles is like that of the pharyngeal muscles. The *crop* is absent in this species. Keilin & Nuttall (1930), Sikora (1916), and Patton & Cragg (1913) have noted its absence from the human louse also. Florence (1921), however, reports its presence in the hog louse, *Haematopinus suis* and Qadri (1948) noted its presence in the buffalo louse

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Fig. 20. Alimentary canal (diagrammatic). *m.t.* mouth tube; *cb.* cibarium; *c.s.g.* cephalic salivary gland; *s.d.* salivary duct; *ph.* pharynx; *oe.* oesophagus; *th.s.g.* thoracic salivary gland; *st.* stomach; *mp.t.* Malpighian tubules; *ins.* intestine; *r.g.* rectal glands; *rect.* rectum.

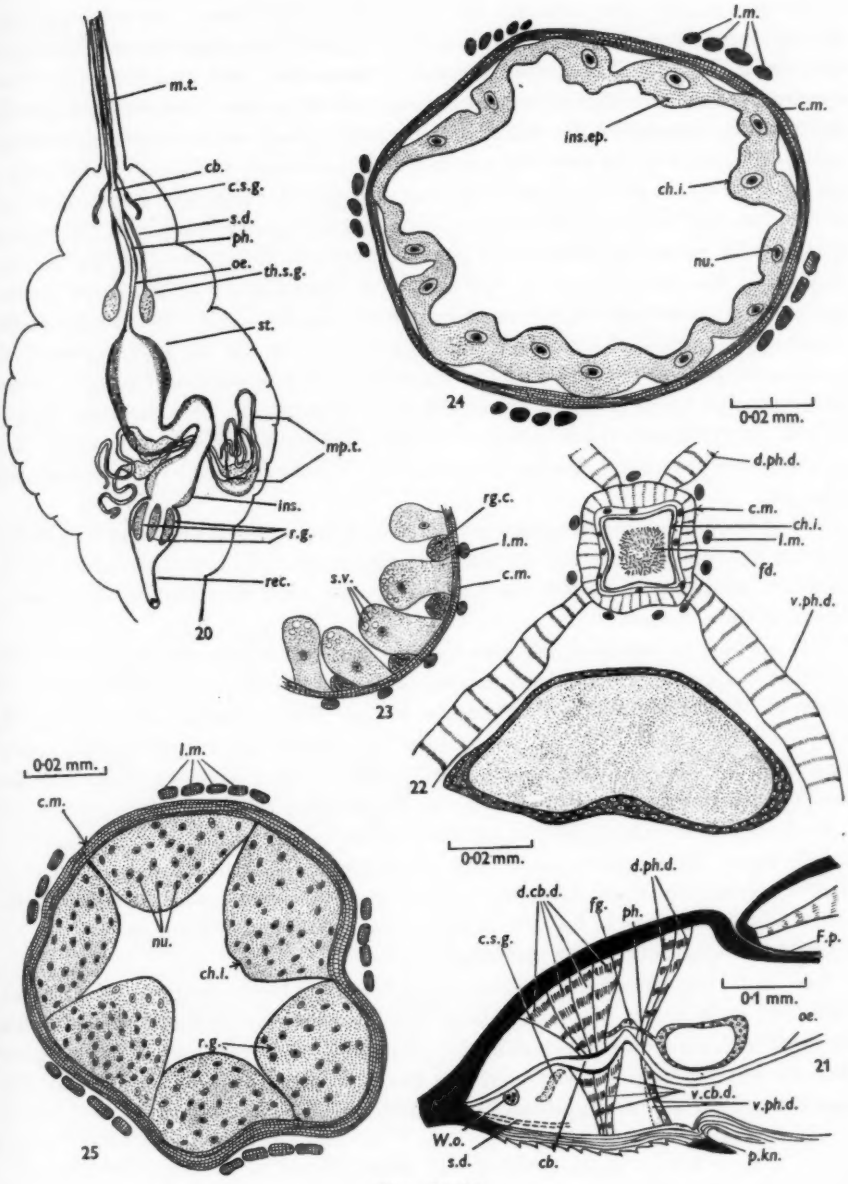
Fig. 21. Longitudinal section of the head. *W.o.* Weber's organ; *s.d.* salivary duct; *c.s.g.* cephalic salivary gland; *cb.* cibarium; *d.cb.d.* dorsal cibarial dilators; *v.cb.d.* ventral cibarial dilators; *fg.* frontal ganglion; *d.ph.d.* dorsal pharyngeal dilators; *v.ph.d.* ventral pharyngeal dilators; *ph.* pharynx; *oe.* oesophagus; *p.kn.* posterior knob; *F.p.* Ferris plate.

Fig. 22. Transverse section of pharynx. *d.ph.d.* dorsal pharyngeal dilator; *v.ph.d.* ventral pharyngeal dilator; *c.m.* circular muscle; *l.m.* longitudinal muscle; *fd.* food contents; *chi.i.* chitinous intima.

Fig. 23. T.S. of stomach (partly shown). *rg.c.* regenerative cell (nest cell); *s.v.* secretory vacuole; *l.m.* longitudinal muscle; *c.m.* circular muscle.

Fig. 24. T.S. of intestine. *nu.* nucleus; *ch.i.* chitinous intima; *ins.ep.* intestinal epithelium; *l.m.* longitudinal muscles; *c.m.* circular muscle.

Fig. 25. T.S. through rectal glands. *r.g.* rectal gland; *chi.i.* chitinous intima; *nu.* nuclei; *c.m.* circular muscle; *l.m.* longitudinal muscles.


m.t.
cb.
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rg.c.
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21
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Figs. 20–25.

H. tuberculatus. Snodgrass (1899) stated that the crop is present in the chewing lice (Mallophaga).

The oesophagus opens directly into the *stomach* and there is no valve guarding the aperture of communication. The stomach is lined by a single layer of nucleated epithelium (Fig. 23). The epithelial layer is surrounded, first by a layer of circular muscles, and, outside this, by a layer of longitudinal muscles. Among the epithelial cells there is a gradation in size and state of activity and on that basis, the mid-gut is divisible into two regions: the anterior and the posterior. The epithelial cells of the anterior region are comparatively large and globular in shape. Their inner ends project into the lumen of the stomach. The shape, size and position of the nucleus of these cells vary according to the physiological activity of the cells. The nucleus may lie in the basal region of the cell or it may migrate to the inner border. Secretory granules and numerous secretory vacuoles occur in the cytoplasm. The process of secretion of ferments by the epithelium of the stomach curiously resembles the process described by Qadri (1948) in *H. tuberculatus*. The inner end of each epithelial cell is loaded with secretion and it separates from the main body of the cell, carrying with it the secretion. No striated border is seen in the cells. Nest cells are present towards the basal region, lying in between two large secretory cells (Fig. 23).

The epithelial cells of the posterior portion of the stomach are more or less flat. They do not contain secretory vacuoles. Probably, they are not concerned with the digestion of food but absorb the food which has been disgested in the anterior region of the stomach.

The opening of the stomach into the intestine is not guarded by valves. The *intestine* of *Haematomyzus* is not thrown into coils like the intestine of *Pediculus humanus*. It is lined by a thin chitinous intima. The epithelial layer of the cells is folded into six raised areas. There is no cell boundary and the cells form a syncytium (Fig. 24). The epithelial layer is surrounded first by a layer of circular muscles, then by longitudinal muscle fibres which are fewer in number. There are little gaps at the bases of the elevated folds, between the epithelial cell layer and the circular muscle layer. These spaces have possibly arisen during early stages of development when the epithelial layer, by a faster rate of growth, was pushed into the lumen of the intestine to become detached from the muscle layer, which did not grow at the same rate as the epithelial layer.

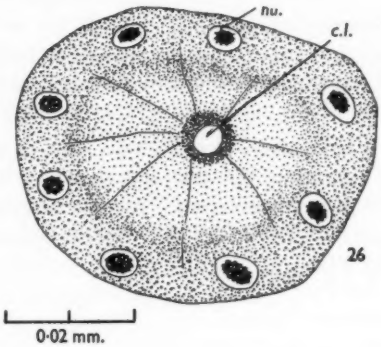
Ries (1931) mentioned the presence of symbionts in the intestine of the elephant louse, but we have not found any there. The intestine ends in the *rectum*, which is provided with six rectal glands (Fig. 20). Histologically, each rectal gland is a cell syncytium; the nuclei are scattered and the cytoplasm is granular (Fig. 25). The *anus* is terminal and is supported by a chitinous *anal ring*.

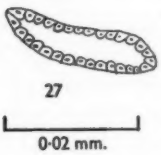
SALIVARY GLANDS

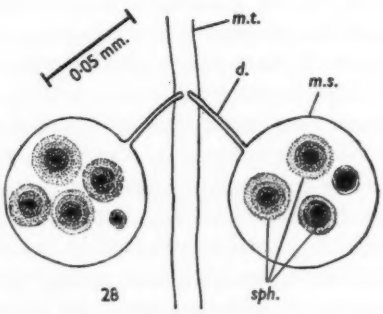
The elephant louse possesses two pairs of salivary glands. One pair is situated in the anterior part of the head, below and each on either side of the cibarial pump. This pair is here termed the *cephalic pair*. The second pair is situated within the thorax, near the anterior region of the meta-thorax, each of them lying

on either side of the oesophagus. For convenience of description this pair is called here the *thoracic pair*. In gross dissection none of the glands shows any pigmentation.

Each of the cephalic pair of salivary glands is tubular and slightly bent (Fig. 20). A very small, narrow duct comes out from the anterior end of each of the cephalic pair of salivary glands, which, after running independently for a short distance in the anterior region of the head, unites with the duct of the thoracic gland of the







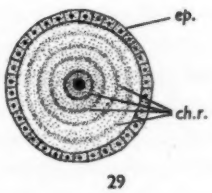


Fig. 26. T.S. of thoracic salivary gland. *nu.* nucleus; *c.l.* central lumen.

Fig. 27. L.S. of cephalic salivary gland.

Fig. 28. Weber's organ. *m.t.* mouth tube; *m.s.* membranous sac; *d.* duct opening into mouth tube; *sph.* spheroids.

Fig. 29. T.S. of a spheroid. *ep.* epithelium; *ch.r.* chitinous rings.

corresponding side. Histologically, each of the cephalic pair of glands has a single layer of thin glandular cells (Fig. 27).

The thoracic pair of salivary glands is relatively large, broad and straight (Fig. 20). A narrow duct, (which is relatively wider than each duct of the cephalic pair of glands) issues from each of the thoracic glands and runs for some distance along the side of the oesophagus, and then passes downwards. When it enters the head, this duct runs below the alimentary canal. It receives, near the base of the

rostrum, the duct from the cephalic gland of the same side. The pair of ducts run parallel to the mouth-tube as far as the tip of the rostrum (Figs. 3, 20). Each duct then opens separately at the terminal end of the rostrum, on either side of the mouth opening. Each opening of the salivary duct is guarded by a thin, inconspicuous chitinous ring.

In transverse sections, the thoracic gland is circular in outline; it has, in the middle, a very narrow lumen. Its cells are tall and the nucleus of each cell is situated peripherally (Fig. 26). The cytoplasm of the cells is granular and takes up a dark blue stain with Delafield's haematoxylin.

The elephant louse is distinguished from the Anoplura, the Mallophaga, and the Psocids by its possession of a cephalic and a thoracic pair of salivary glands. In the Anoplura and Mallophaga two pairs of salivary glands are situated within the thorax, but none are present in the head region. The elephant louse is also remarkable in its possession of a paired opening of the salivary glands, instead of the single opening of a common duct on the hypopharynx. It may be remarked that Finlayson (1949) has reported double openings of the salivary glands in some Psocoptera. This can be regarded as a primitive character.

WEBER'S ORGAN

In 1938, Weber called attention to the presence of a peculiar 'neues Organ' within the anterior region of the head of the elephant louse. He described it as an aggregation of round cuticular bodies, contained in a sac and attached to the roof of the head by a membranous connexion. But he did not go into the histological details of the new organ. He, however, discussed the possibility that this organ may be homologous with the 'Palménschen Organ' of Ephemerids or with the *corpora allata* of *Bacillus rossii*, or with the ventilator apparatus, or that it is a static organ. Because the existence of such bodies has not been recorded before in any species of insect, we call this organ *Weber's organ* after the name of its discoverer, notwithstanding the confusion about its function. We give here its histological peculiarities.

Weber's organ consists of two groups of spheroid bodies. Each group is contained within a sac, which lies on either side of the mouth-tube in the anterior region of the head (Fig. 28). The number of the spheroids in each group varies from two to five. These spheroids are intimately massed together so as to give the impression that a single chitinoid body is present within each sac.

The wall of each enveloping sac is very thin and appears membranous. Each sac seems to give out a duct-like, fine strand, which is exceedingly thin, and this seems to open dorsally into the mouth-tube. Each spheroid body is seen through the transparent wall of the membranous sac as a dark solid body. Each spheroid is hard and, when it is detached from the enclosing sac in course of dissection, it sinks down in water. In sections it is seen that each spheroid arises as a deposit of chitinous substances at the centre, with a secondary deposit of chitinous material around it (Fig. 29). For this reason, in microscopical sections, each spheroid appears to consist of concentric rings in a chitinous mass. Each spheroid is surrounded by a thin epithelial layer, the cells of which are flattened (Fig. 29). This epithelial layer is more or less similar to the hypodermis in cellular structure; it

seems to be responsible for the secretion and deposition of the chitinous mass. Weber (1938) reports that these chitinoid bodies are absent in the few exuviae which he examined. Evidently the chitinoid bodies are not cast out during ecdysis, and so they are not seen in the exuviae. They remain within the body and deposition of the secondary layer of chitin around the central chitinoid mass occurs as growth proceeds after each ecdysis. This explains the variable size of the spheroids, the range of variation, being from a small body with only a central mass of chitinoid deposit to a large body with several layers of secondary deposit of chitin around the central mass. Presumably, the number of secondary rings indicates the growth period.

From the existence of the epithelial cells around the chitinoid mass, it seems that the spheroids are secretory products liberated by the enclosing epithelium. Since the hard chitinous balls cannot pass into the mouth-tube, the duct being too narrow for their passage, it is more probable that each is an excretory product eliminated by the enveloping epithelial cells and stored within the body. The number and size of the spheroid bodies probably depend upon the physiological activity of digestion. We have failed to note the connexion, mentioned by Weber (1938), between these bodies and the dorsal integument of the head.

RESPIRATORY SYSTEM

The respiratory system does not differ greatly from that of the sucking lice. There are seven pairs of spiracles, six pairs on the abdomen and one pair on the thorax. The abdominal spiracles are placed laterally between the pleurites and lateral tergites of the second to the seventh segment.

The first pair of spiracles is, as Ferris (1931) pointed out, situated on the meso-thorax and not on the prothorax as Webb (1946) claimed. Each mesothoracic spiracle opens ventrally at the lateral border of the thorax, above the coxa of the meso-thoracic leg. Its opening is circular in outline and is surrounded by a broad peritreme. It leads into the atrium, which is nearly spherical, and not cylindrical as Webb (1946) stated. The inner surface of the atrium is interspersed with hairs and spines. As Webb (1946) noted, the apex of the spine is expanded. The internal aperture of the atrium is provided with two chitinous rods, which approximate each other. An occlusor muscle passes from the ventral wall of the integument to the dorsal wall of the atrium, slightly above the junction of the latter with the trachea (Fig. 30). The abdominal spiracles open dorsally at the lateral margins in between the pleurites and the lateral tergites of the second to the seventh segments. The abdominal spiracles differ from the thoracic ones in important points. They open dorsally, while the thoracic pair opens ventrally. The atrium is funnel-shaped. The internal surface of the atrium is covered with sharp, pointed, minute hairs, but the large blunt spines noted in the atrium of the thoracic spiracle are absent (Fig. 31). We could not find the occluding apparatus and the lever in it mentioned by Webb (1946). Webb (1946) also mentions the presence of a gland opening dorsally by a fine canal into the atrial wall, as in *Haematopinus*, and on that basis, he suggests a close relationship between *Haematopinus* and *Haematomyzus*. We have not found any such gland. The tracheal system of *Haematomyzus* is composed

of two main pairs of longitudinal tracheae with a single pair of spiracular branches in the thorax and five pairs in the abdomen. There is no sexual dimorphism in the disposition of tracheae, which is shown in the Fig. 32.

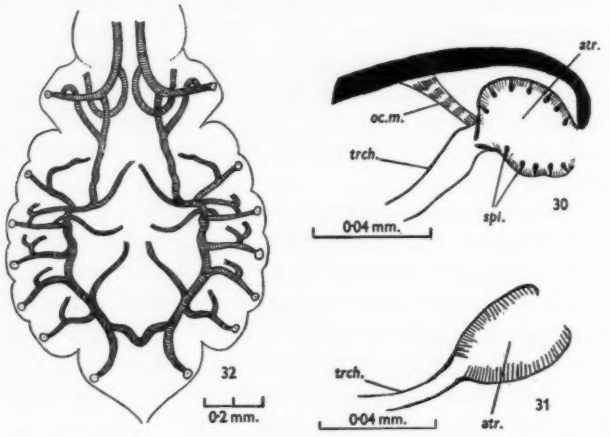


Fig. 30. L.S. of the thoracic spiracle. *atr.* atrium; *trch.* trachea; *oc.m.* occlusor muscle; *spi.* spines.

Fig. 31. L.S. of abdominal spiracle. *atr.* atrium; *trch.* trachea.

Fig. 32. Tracheal system of male.

REPRODUCTIVE SYSTEM

Female

The ovarioles are five in number on either side of the body and the ovary is polytrophic of the meroistic type. The terminal filament is short and ends freely within the body cavity. As in the Anoplura, there are six undifferentiated oogonia at the early stage of development of the egg. Later, these are transformed into nurse cells, which are five in number, and the oocyte, which is single. The nurse cells degenerate as the oocyte matures and they ultimately disappear. Each of the epithelial cells surrounding a growing oocyte has two to four nuclei. Later on, the epithelial cells diminish in thickness and become flat and each cell contains two nuclei (Fig. 34). Murray (1926) observes that the bi-nucleate condition arises by mitotic division of the single nucleus, so that the nuclear and nucleolar surfaces are increased during the phase of intense secretory activity. The occurrence of bi-nucleate-follicular epithelium in the elephant louse and also in the Mallophage is remarkable, because it does not occur in the Anoplura, in which group each follicular epithelial cell contains a single nucleus (Ries, 1932).

The chorion around the egg is of considerable thickness and has a firm consistency. Evidently it is secreted by the follicular epithelium.

At the posterior end of the egg a very characteristic structure is noticeable. Graber (1872) applies the term 'Eistigma' to the similar structure in Anoplura. The egg-stigma consists of many fine radiating canals, each canal running in between two slender hair-like epithelial cells. The fine canals, together with the bounding

cells, penetrate deep and reach the egg-mass. The chorion disappears from the region where the radiating canals were developing (Fig. 34). The accompanying epithelial cell, running on either side of each canal, is binucleate, like the follicular epithelium. The inner end of this cell is drawn out into a fine thread, while the outer end remains broad. The canals are presumably formed as follows: the follicular cells at the posterior pole of the ovum become narrow, slender and elongated. The cells move inward and then separate from each other, leaving a fine canal in between. Graber (1872) regarded the canals as passages for entry of air into the egg. Leuckart (1855) considered that they form an adhesive apparatus for fixing the egg to the hair of the host. Florence (1921) believed that they served for the passage of symbionts. Ries (1932) did not accept any of these views, but he could not decide what their function was. We strongly believe that these canals are the micropyles.

The ovarioles, on either side, open into an oviduct, which is very short (Fig. 33). The oviduct is composed of a single layer of tall, cylindrical cells, which have distinct nuclei and granular cytoplasm. There is no chitinous intima in the duct, a fact which suggests that the duct is of mesodermal origin (Fig. 36). These cells are highly glandular and secrete adhesive substances for attachment of eggs to the hair of the host. The accessory gland found by Florence (1921) and Qadri (1948), in the Anoplura, has not been found in *H. elephantis*. The two oviducts open separately into the median vagina (Fig. 33) which is relatively large and accommodates the large egg, which stays within the vagina for a short period before its emission. The epithelial cells of the vagina are uninucleate and have a thin chitinous lining. The vagina opens into the female genital opening, which lies on the ventral side of the seventh abdominal segment.

The spermatheca as reported by us (1951) is a round sac with a short neck. The neck leads into a long duct which opens on the dorsal side of the vagina, the opening being marked by a sclerotic scar, as noted by Mjöberg (1910) in *Acanthospinus sciurinus*. The lumen of the spermatheca is lined by a thin chitinous intima. The intima within the spermathecal duct is thick. No spermathecal gland occurs. It is probable that, because there are no glands, the spermatophores are transferred directly into the spermatheca, as we (1951) found in *Linognathus vituli*. The musculature in the spermatheca is feebly developed. The external genitalia of the female consists of a pair of valves. Each valve has a broad posterior blade, which is slightly curved, and a narrow apodeme that lies in the seventh segment (Fig. 37). The posterior portion of the blade is provided with numerous long setae.

Male

The male reproductive organs are similar to those of the Anoplura. There is a pair of testes situated on each side of the fourth segment of the abdomen. The two testes of each side remain closely apposed to one another. Each testis is more or less cordate in shape and consists of two follicles. Each follicle is cylindrical and four zones in it are discernible. The apical zone or the germarium is followed by a middle zone of growth, where sperm mother-cells are formed. In the next zone

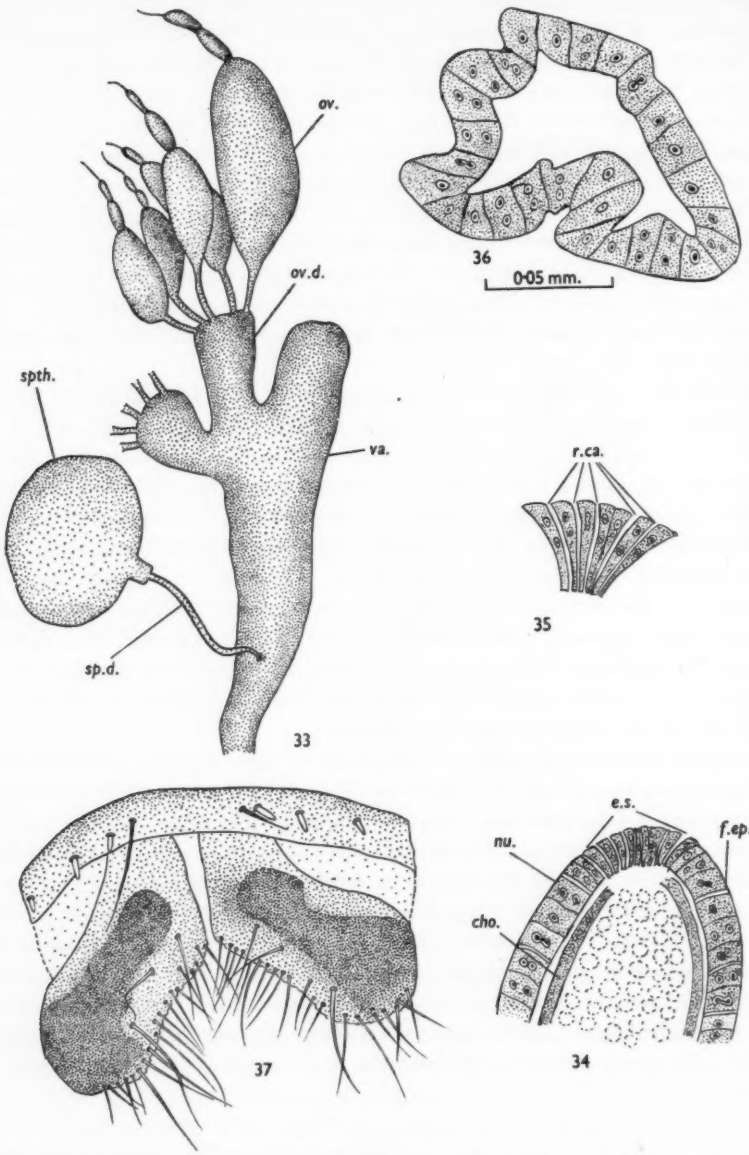


Fig. 33. Female reproductive system. *ov.* ovariole; *ov.d.* oviduct; *va.* vagina; *spth.* spermatheca; *sp.d.* spermathecal duct.

Fig. 34. L.S. of ovariole. *nu.* nucleus; *cho.* chorion; *f.ep.* follicular epithelium; *e.s.* egg-stigma.

Fig. 35. Egg-stigma magnified. *r.ca.* radiating canals.

Fig. 36. T.S. of oviduct.

Fig. 37. Female genitalia.

reduction division occurs and the sperm mother-cells develop into spermatids. The last zone is the zone of transformation into spermatozoa. Each testis gives out an extremely short vas efferens. The vasa efferentia of each side soon unites to form the vas deferens. The vas deferens is a long tube; it is lined by a single layer of epithelium. The muscle elements around the vas deferens are few in number (Fig. 39).

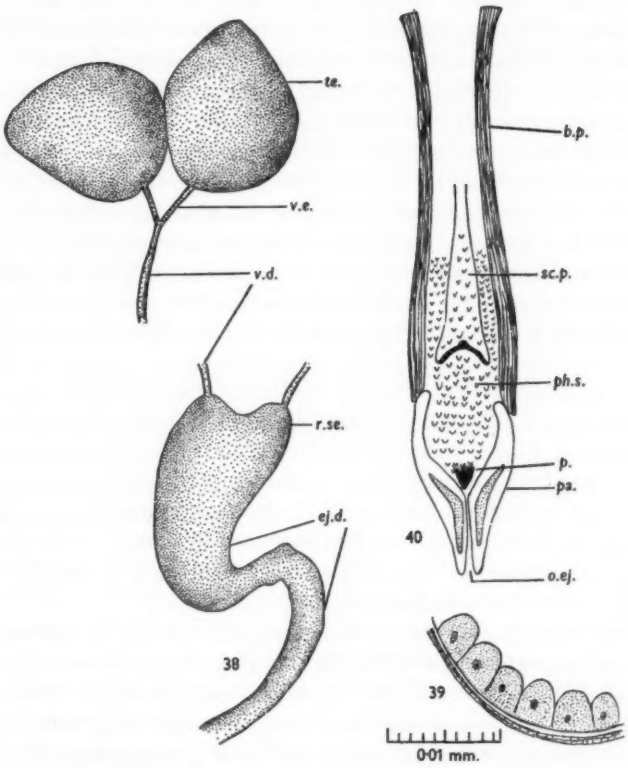


Fig. 38. Male reproductive system. *te.* testis; *v.e.* vas eferens; *v.d.* vas deferens; *r.se.* receptaculum seminis; *ej.d.* ejaculatory duct.

Fig. 39. T.S. of vas deferens.

Fig. 40. Male genitalia. *b.p.* basal plate; *sc.p.* sclerotic plate; *ph.s.* phallic sac; *p.* penis; *pa.* paramere; *o.ej.* orifice of ejaculatory duct.

The vas deferens opens separately into the dilated receptaculum seminis. The receptaculum seminis in the elephant louse is, unlike that of the Anoplura, single (Fig. 38). It possesses at its posterior end, glandular epithelium, the secretion of which possibly helps in formation of the spermatophores. We regard this glandular portion of the receptaculum seminis as equivalent to the accessory gland of other insects. The glandular portion of the tube opens posteriorly into the ejaculatory duct. The ejaculatory duct is divided into anterior and posterior portions (Fig. 38).

The anterior portion is wide and coiled. The posterior portion is narrow and long. It communicates with the phallic sac through an aperture that is placed near the base of the basal plates.

The male genitalia consist of a basal plate, a pair of parameres, a single median phallic sac, and a sclerotic plate. The basal plate is the largest of all the components of the male genitalia. The space between the two arms of the basal plate is narrower at the anterior, and wider at the posterior region. At the hind end of the basal plate, there is a pair of parameres. The two parameres at the posterior end approximate each other, so that they form a very narrow passage (Fig. 40). The tips of the parameres are, as Ferris (1931) stated, sharply curved upwards.

Ventral to the parameres and connected with the basal plate, there is a well-developed, tough, sac-like structure beset with numerous small sclerotic denticles which were called placoids by Ferris (1931). A sclerotic plate is placed on the anterior end of this sac. The hind end of this sac passes into the small sclerotized penis (Fig. 40). In Anoplura, a similar sac has been variously termed the 'preputial sac' by Mjöberg (1910), the 'mesosome' by Cummings (1916), the 'endomere' by Ewing (1932) and the 'vesica penis' by Nuttall (1917). In view of the connexion of the sac with the penis and of its probable function, we call it the *phallic sac.*

DISCUSSION ON THE SYSTEMATIC POSITION

The systematic position of the elephant louse has been the centre of controversy since its discovery. Piaget (1869), who was unaware of its anatomical peculiarities, put it in the Anoplura. Ferris (1931), in consideration of the biting mouthparts, created the suborder Rhyncophthirina of the Mallophaga, in order to accommodate *Haematomyzus*. Weber (1939*a*, *b*) created the order Phthiraptera of the super-order Psocoidea and he treats the Rhyncophthirina together with the Mallophaga and Anoplura, as a suborder of Phthiraptera.

The elephant louse in structure and in the disposition of its alimentary canal, the polytrophic ovary and the double follicular testis, seems allied to the Anoplura. On the basis of the spiracular structure, Webb (1946) claims that a close relationship exists between *Haematomyzus* and *Haematopinus* and he suggests the inclusion of the elephant louse in the family Haematopinidae of the Anoplura. Hopkins (1949), however, disputes Webb's claim on the ground that undue stress has been laid by Webb on the spiracular structure.

As Ferris (1931) showed, the elephant louse also has Mallophagan features. The gonapophyses of *Haematomyzus* resemble those of the Trichodectidae (Webb, 1946). Moreover, in contrast to the uninucleated condition of the follicular epithelium of the Anoplura, both *Haematomyzus* and Mallophaga possess binucleated follicular epithelium in the ovary. It is interesting to recall that Webb (1946), in support of his claim for the inclusion of the elephant louse in the Anoplura on the basis of the spiracular structure, discards Ferris's conclusion by reasoning that the mouthparts and the legs, on which Ferris based his conclusion, are just those regions most likely to be affected by environmental changes and these, therefore, are least reliable as a guide to taxonomy. Moreover, the value of the mouthparts as a guide to the separation of Anoplura and Mallophaga seems overestimated in view of

Clay's (1949) claim that piercing stylets are also present in some members of the Mallophaga.

In view of the differences both from the Anoplura and the Mallophaga, and on account of retention of primitive characters, such as the existence of the genital opening on the seventh abdominal segment, the double opening of the salivary glands and the possession of special features, such as Weber's organ and the cephalic and thoracic salivary glands, we favour complete separation of the elephant louse, from both the Anoplura and the Mallophaga. We suggest the creation of a new order Rhyncophthiraptera to accommodate the family Haematomyzidae, the sole representative of which is *Haematomyzus elephantis* Piaget.

The origin of the louse from a psocoidean ancestor suggested by Weber (1939*a*, *b*) seems reasonable. That ancestor probably lived in the crevices and cracks of trees and rocks and fed on animal and plant detritus. Later, it migrated to birds, due to nearness of habitat and accessibility of animal detritus, which may be feathers of birds. Further, migration of the psocoidean descendant to the elephant might have occurred during the elephant's endeavour to groom itself against the rough surfaces of trees and rocks. Isolated thus on the animal host, this louse eventually became a separate species, the elephant louse. The persistence of a few ancestral characters, notwithstanding isolation, may account for certain similarities between its characters and those of the biting and sucking lice. Rotation of the mandible to 180°, and its disengagement from the oral aperture, induced adaptive changes in function, biting changing to fixation on to host's skin. These changes followed the change from free-living to ecto-parasitic existence, and explain the changes in the direction of line of evolution of the Psocids.

SUMMARY

Detailed accounts of the morphology of the elephant louse and of its phylogenetic affinities and origin are given. The main points noted are: The peculiarity of the knobs and plate in the head are described. The mouthparts are described, a notable feature being the rotation of the mandible to 180 degrees. Structural peculiarities of the thorax with the muscles are given. The abdomen is found to consist of eight segments. The alimentary canal is described; no crop has been found. The histology of Weber's organ is given, and it is held that this organ is excretory in function. The structure of the spiracle is described, no spiracular gland has been found. The reproductive organs are described. In the female, the follicular epithelium of the ovary is binucleate and a spermatheca is present. In the male, the testis is bi-follicular. The affinities of the elephant louse are discussed and the creation of a new order, the Rhyncophthiraptera, to accommodate *Haematomyzus*, is suggested.

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ON THE INVASION OF THE CENTRAL NERVOUS SYSTEM BY NEMATODES

I. THE INCIDENCE AND PATHOLOGICAL SIGNIFICANCE OF NEMATODES IN THE CENTRAL NERVOUS SYSTEM

By J. F. A. SPRENT

University of Queensland Veterinary School, Brisbane, Queensland

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INCIDENCE

Nervous symptoms have been frequently associated with nematode infections in man and animals, but in relatively few instances have nematodes been recovered from the tissues of the central nervous system. The earliest reference appears to be that cited by Rudolphi (1808, p. 131) in which Du Verney *c.* 1706 found a roundworm in the superior longitudinal sinus of the brain of a child. Since then they have been sporadically reported from the central nervous system and its associated structures, sometimes in apparently normal animals, but also in animals which have manifested nervous symptoms and following experimental infection. This paper is an attempt to review the available literature on this subject and to assess the pathological significance of the various nematode species which gain access to the central nervous system.

(i) *Ascaridoidea*

Fülleborn (1921, 1925) and Hoeppli (1923) observed larvae of *Ascaris suum* and *Toxocara canis* in the brain following experimental infection. Yokogawa (1923) observed haemorrhages on the brain and meninges after experimental infection with *T. canis*, but after infections with *Ascaris lumbricoides* there were only submiliary haemorrhages. Yamaguchi (1925) observed the larvae of *A. lumbricoides* in the brain tissues, blood vessels, ventricles and chorioid plexus of guinea-pigs after experimental infection. Tiner (1949, 1951, 1953*a*, *b*) demonstrated that symptoms of nervous impairment occur in various species of rodents following experimental

infection with the larvae of *A. columnaris* from the raccoon, skunk and badger. He compared the growth of these larvae in the brain, and relating their growth rate and maximal size to the pathological effects of infection, he found that raccoon ascaris larvae grew more rapidly and attained a larger size than the others and consequently were the most pathogenic. He showed that raccoons could be infected with this parasite through the ingestion of infected mice, and suggested that damage to the central nervous system increases the probability that the intermediate host will be eaten by the final host. Beaver, Snyder, Carrera, Dent & Lafferty (1952) experimentally infected mice with the eggs of *Toxocara canis* and *T. mystax*; they recovered living larvae from the brain. Sprent (1953) recovered living larvae of *Ascaris columnaris*, *Toxascaris transfuga* and *Toxocara canis* from the brain of mice after experimental infection. Beautyman & Woolf (1951) found an ascaris larva in the thalamus of a child who had died of poliomyelitis; the larva was identified as *Ascaris lumbricoides*, although the possibility was pointed out that the larva might be an ascaridid from a dog or cat. Mozgovoi (1953) cites Dubinin as finding the larvae of *Ascaris tarbagan* in the brain of experimentally infected mice.

(ii) *Filarioidea*

Abilgaard (quoted by Rudolphi, 1809, p. 63) found *Setaria equina* in the subdural space of a horse. Von Linstow (1883), in reviewing a book by Perroncito, referred to the larvae of *S. cervi* entering the cranial cavity. Railliet & Moussu (1893) found lesions in the spinal cord of a paralysed donkey which they attributed to *Parafilaria multipapillosa*. Wyman (1868) and Cahall (1889) found filarial nematodes, *Filaria anhingae*, between the cerebellum and the cranium of snake-birds. Place (1911) found adult filariae, probably *Setaria* spp., in the spinal cord and meninges of horses, cattle and buffaloes; he concluded that they caused the softening and congestion of the spinal cord characteristic of the disease known as 'Kumree'. Peruzzi (1928) found adult filariae in the brains of five monkeys which had died after experimental trypanosomiasis; the parasites were interlaced as a network in the subarachnoid space in the region of the brain stem; microfilariae were recovered from the cerebro-spinal fluid. Tha Mya (1928) recovered microfilariae from the cerebro-spinal fluid of a human patient who died after manifesting cerebral symptoms. Wetzel & Enigk (1936) recovered a filaria from the subdural space in the dorsal region of the spinal cord of an elk, but gave no specific determination as the parasite was immature. Hashimoto (1939) found microfilariae of *Dirofilaria immitis* associated with congestion and eosinophilia in the pituitary gland of dogs; Dacorso (1944) found these microfilariae in the brain of a dog which had shown symptoms of rabies. Schwangart (1940) found filariae in various stages of maturity, possibly *Seteria cervi* or *Onchocerca flexuosa*, in the subdural space of the brain and spinal cord of deer; he concluded that they were responsible for the paralytic symptoms which he had observed in the infected animals.

Innes (1951) and Innes, Shoho & Pillai (1952*a*, *b*), from observations on natural and experimental infections, concluded that 'lumbar paralysis' of goats which has been known in Japan and Ceylon for many years, resulted from invasion of the central nervous system by larvae of *Setaria digitata*. Ishii, Yajima, Sugawa,

Ishiwara, Ogata & Hashiguchi (1953) inoculated goats subcutaneously with larvae of *S. digitata* dissected from infected mosquitoes. Seven out of nine infected goats exhibited nervous symptoms, while ten untreated controls remained healthy; in two of the infected goats, nematodes were recovered from the nervous system. Bush (1951) inoculated thirty goats with 300 infective larvae of *S. digitata* dissected from mosquitoes, but obtained negative results. Referring to a similar disease of goats in Ceylon, Innes & Shoho (1952) write: 'McGaughey (1951)—at our instigation—subsequently demonstrated the causal setaria'. This conclusion is open to doubt judging by the photographs presented in McGaughey's paper (1951).

Kennedy, Whitlock & Roberts (1952) found *Neurofilaria cornellensis* Whitlock 1952 in sections of the brain and spinal cord of two lambs suffering from paralysis; they have proposed the name 'neurofilariosis' for the disease which they believe is caused by this parasite.

Lynd & Short (1952) found microfilariae lying freely in the brain tissue and subarachnoid space of a skunk.

(iii) *Trichuroidea*

Borell (1875) reported that he had found trichina larvae in the blood vessels of the dura mater of a raven; Virchow commented that they were possibly microfilariae. Larvae of *Trichinella spiralis* have frequently been found in the spinal fluid in human cases of trichinosis (Van Cott & Lintz, 1914; Bloch, 1915; Cummins & Carson, 1916; Lintz, 1916; Elliott, 1916; Salan & Schwartz, 1928). They were found in brain tissues in fatal human trichinosis by Frothingham (1906). Sicard (1914), Hassin & Diamond (1926) and Most & Abeles (1937).

(iv) *Strongyloidea*

Price (1928) described a case of paralysis in a pig resulting from pressure on the spinal cord by a caseous lesion containing *Stephanurus dentatus*. Raffensperger (1932) also found this parasite in the vertebral canal of pigs. Neumann (1892) stated that a strongyle was recovered by Albrecht (1872) from the cerebellum of a horse; death was preceded by violent nervous symptoms and there were severe inflammatory changes in the brain. Von Heill (1874) found an almost mature strongyle in the right cerebral hemisphere of a horse which had shown repeated attacks of excitement. There was an abnormal amount of fluid in the ventricles and congestion of the blood vessels.

(v) *Metastrongyloidea*

Capdebielle & Hussenet (1911) reported a case of a dog which died after a series of epiliptiform attacks; a living larva of *Angiostrongylus vasorum* was found within a haemorrhagic lesion of the left cerebral hemisphere. Wolffhügel (1934) described a disease of cats in South America, characterized by slowly advancing paralysis, caused by distension and thrombosis of the veins in the subarachnoid space in the lumbar part of the spinal cord resulting from the presence of eggs and mature forms of *Gurltia paralysans*. Burg, Baudet & Verwey (1953) found mature specimens of a nematode, tentatively identified as *Protostrongyloides cervi*, in the subarachnoid space of deer; in one case they were associated with a large haemorrhage in the

cranial cavity. The present writer (unpublished) has found larvae of *Aleurostrongylus abstrusus* in the macerated brain of an apparently normal cat. Mackerras & Sandars (1954) found that the larvae of *Angiostrongylus cantonensis* when fed to rats, migrate into the cerebral hemispheres and pass thence to the lungs via the venous system.

(vi) *Rhabditoidea*

Fülleborn (1921) and Yamaguchi (1925) demonstrated that the larvae of *Strongyloides stercoralis* reach the brain of experimentally infected animals.

(vii) *Dioctophymatidae*

Von Brand & Cullinan (1943) demonstrated that the larvae of *Eustrongylides ignotus*, when transferred from the tissue of freshwater fish and implanted in the subcutaneous tissues of rats and chickens, migrated into the central nervous system and caused extensive and fatal damage. Rudolphi (1808, p. 131) suggested that the worm found by Du Verney in the brain of a child might be *Dioctophyme renale* (cf. Introduction).

(viii) *Nematodes of uncertain identity*

Rathke (1837) found small nematodes in vesicles in the brain of embryonic *Lacerta agilis*. Diesing (1851, p. 342) lists *Nematoideum rajae batis* Rudolphi, originally found by Monro in vesicles on the ophthalmic branch of the trigeminal nerve of a ray. Bunzl (1904) observed encapsulated larval nematodes in the brain of a mole (*Talpa europea*); they were situated in various parts of the brain, expecially in the corpus callosum and internal capsule. Widakowich (1905) observed sexually mature nematodes in the connective tissue between the dura mater and the hypophysis of a cat. Fenstermacher (1934) found an immature nematode in the brain stem of a moose (*Alces americanus*) showing symptoms of inco-ordination; no specific determination was given.

PATHOLOGICAL EFFECTS OF NEMATODES ON THE CENTRAL NERVOUS SYSTEM

(i) *Effects attributed to nematodes situated outside the central nervous system*

It has long been held that nematodes in the intestinal tract may give rise to nervous symptoms. Rudolphi (1808, p. 470) gave a formidable list of such symptoms, and others have been added since, e.g. verminous epilepsy (Boissière 1886), brain symptoms (Gibson, 1907), parasitic epilepsy (Pagniez & Plichet, 1938), helminthic meningism (Zau, 1935) and reflexive epilepsy (Robbio, 1946). In these reports there is no evidence that parasites had invaded the central nervous system, and in most instances the symptoms disappeared after anthelmintic treatment. Nervous symptoms have also been described in somatic nematode infections such as human trichinosis (Pohlmann, 1942; Gould, 1945, pp. 215–19), human filariasis (Brunetière, 1913, cited by Bertrand-Fontaine, Schneider, Wolfromm & Cagnard, 1948; Kenney & Hewitt, 1950) and canine filariasis (Daschoff & Novy, 1945).

There is at present no satisfactory explanation for these nervous symptoms. The evidence for the occurrence of toxins in trichinosis has been reviewed by Gould (1945), and in ascaris infections by Sprent (1949). There appears to be insufficient

evidence to indicate that toxins are elaborated by these parasites. On the other hand, there is experimental evidence to indicate that infection with the larvae of *Ascaris suum* induces a state of hypersensitivity whereby allergic symptoms are manifested on secondary exposure to injected antigens (Sprent, 1950, 1951). Rachmanow (1914) demonstrated changes in the central nervous system resulting from injection of ascaris extracts in previously sensitized animals, hence it is possible that changes in the nervous system may accompany allergic reactions brought about by ascaris antigens. Referring to ascariasis, Craig & Faust (1940, p. 297) state: 'In persons sensitive to absorbed foreign proteins, generalized toxemia or specific nervous symptoms, such as insomnia, twitching and restlessness, and even manifestations simulating meningitis and paraplegia may result.' Lotmar (1951) described two human cases with ascaris infestation of the intestine showing focal epileptiform symptoms. He suggested that a local sensitization of the central nervous system had arisen through the death of migrating larvae in the brain, and that the symptoms arose from exposure of the central nervous system to toxic substances absorbed from the worms in the intestine. To lend weight to this hypothesis, he cited a case where a positive skin reaction with ascaris antigen provoked cerebral symptoms.

(ii) *Direct effects of nematodes on the central nervous system*

Reports on the pathological effects of nematodes in the central nervous system show that there is considerable variation in the changes observed. These changes fall into three categories: (1) haemorrhagic, (2) degenerative, and (3) proliferative. The variation is probably determined partly by the location, partly by the size and partly by the activity of the parasite.

Haemorrhagic changes occur when nematodes arrive in the brain via the arterial circulation, e.g. the superficial haemorrhagic foci described in experimental ascariasis of rodents (Yamaguchi, 1925) and the perivascular haemorrhages in the brain in cerebral trichinosis (Hassin & Diamond, 1926; Most & Abeles, 1937). Nematodes moving through nervous or meningeal tissues may lacerate blood vessels and cause severe haemorrhages, e.g. *Angiostrongylus vasorum* (Capdebielle & Hussenet, 1911) and *Protostrongyloides* spp. (Burg *et al.* 1953).

Degenerative changes are conspicuous in neurofilariasis, being described as tortuous malacic foci (Innes *et al.* 1952*b*), haemorrhagic softening (Ishii *et al.* 1953) and 'inflamed sinuous lines' (Railliet & Moussu, 1893). Degenerative changes are also evident in swelling of the axis cylinders and zones of demyelination (Kennedy, Whitlock & Roberts, 1952). Innes *et al.* (1952*b*) conclude that the lesions produced in the spinal cord by filariae are the result of mechanical trauma caused by the migrating parasites; the lesions were connected with the pia by streaks, indicating that the parasites had penetrated by direct migration from adjacent tissues.

It is probable that the traumatic effect of nematodes in the central nervous system is directly proportional to the size of the parasite. The relatively small larvae of *Toxocara canis* may wander in the brain without causing sufficient damage to provoke symptoms, while larger larvae such as *Ascaris columnaris* and the filarial nematodes do sufficient damage during their migrations to cause definite

Table 1. *Measurements of nematodes found in the central nervous system of birds and mammals*

Parasite	Authority	Host	Location	(Width (mm.)	Length (mm.)
Ascaris columnaris (larva)	Tiner	Mouse	Brain	—	Up to 1·4 (skunk strain) Up to 1·6 (raccoon strain)
Toxocara canis (larva)	Beaver *et al.*	Mouse	Brain	0·018–0·022	0·375
Ascaris lumbricoides (larva)	Beautyman & Woolf	Man	Brain	0·015–0·020	0·330–0·350
Setaria cervi (?)	Schwangart	Red deer	Subdural space	—	23–50
S. digitata		Goat	Spinal cord	0·110 (sheep)*	14 (Ishii *et al.*)
Parafilaria multipapillosa	Railliet & Moussu	Donkey	Spinal cord (?)	Female 0·42–0·44 Male 0·26–0·28	40–56 28
Neurofilaria cornellensis	Whitlock	Sheep	Brain and spinal cord	0·132–0·338	'At least 20 mm.'
Dirofilaria immitis (Microfilariae)	Dacorso	Dog	Brain	—	0·18–0·20
Microfilariae	Lynd & Short	Skunk	Brain	0·004	0·105
'Nematode'	Fenstermacher	Moose	Brain (pons)	0·192–0·238	—
Gurltia paralysans	Wolffhügel	Cat	Spinal cord (subarachnoid space)	Female 0·144–0·155 Male 0·050–0·102	20·5–23·0 12
Aleurostrongylus abstrusus (larva)	Sprent	Cat	Brain	0·022	0·342
Protostrongyloides sp.	Burg *et al.*	Deer	Brain and spinal cord (subarachnoid space)	Female —	55
Trichinella spiralis (larvae)	—	Man	Brain	Male 0·100 0·004–0·005 Hassin & Diamond)	30 0·100 (Most & Abeles)
T. spiralis (?) (larvae)	Borell	Raven	Dura mater	0·004	0·130
Eustrongylides ignotus	von Brand	Rat	Spinal cord	—	60–80
Dioctophyme renale?	Rudolphi	Man	Brain	—	'Circiter quadripollicarem'
Nematode larvae	Bunzl	Mole	Brain	0·015–0·018	0·2 (approx.)
Nematode	Widakowich	Cat	Hypophysis	0·4 (approx.)†	—
Nematode	Cahall	Snake-bird	Subdural space	—	20–40

* Obtained by measurement of fig. 8*c* (Innes *et al.* 1952).
† Obtained by measurement of fig. 3 (Widakowich, 1905).

derangement of the nervous system. The larvae of *Eustrongylides ignotus* migrating into the spinal cord of rats (von Brand & Cullinan, 1943) caused destruction of entire nerve tracts. According to von Brand (personal communication) these nematodes measured 6–8 cm. in length.

Proliferative changes may be diffuse or focal. The diffuse infiltrations include (1) the perivascular infiltrations described by Dacorso (1944) and Kennedy *et al.* (1952) in neurofilariasis, by Hassin & Diamond (1926) in cerebral trichinosis, and by Beautyman & Woolf (1951) in cerebral ascariasis, and (2) the meningeal infiltrations described in neurofilariasis by Innes *et al.* (1952*b*) and Ishii *et al.* (1953). The focal infiltrations usually consist of granulomatous cellular clusters in the vicinity of the parasite. In some instances the cellular reaction was found to consist mostly of glial proliferation, as in trichinosis (Hassin & Diamond, 1926) and neurofilariasis (Kennedy *et al.* 1952). In other instances the cells were mostly of mesenchymal origin, as described in another case of trichinosis by Most & Abeles (1937) and in cerebral ascariasis by Beautyman & Woolf (1951). The larvae found in the mole's brain by Bunzl (1904) were densely encapsulated.

In contrast, certain nematode infections of the central nervous system show no evidence of cellular reaction in the vicinity of the parasite, e.g. in experimental ascariasis of rodents (Hoeppli, 1923; Yamaguchi, 1925) and in the case described by Fenstermacher (1934). It is probable that degenerative and cellular changes in the vicinity of the parasite only appear if the parasite has become quiescent before fixation. If the parasite is moving at the moment of fixation it may lie in apparently normal tissue, while extensive damage may be found in other parts of the central nervous system.

In some instances the nematodes are found in the meningeal spaces. In this location they may cause no effect on the central nervous system, as in the infection described by Cahall (1889), or they may produce severe nervous symptoms. These may result by (1) compression on the spinal cord, as may occur with *Stephanurus dentatus* (Price, 1928), (2) by distension and thrombosis of the blood vessels of the spinal cord as in infections with *Gurltia paralysans* (Wolffhügel, 1934), (3) by destruction of the meninges as described by Schwangart (1940), or (4) by meningeal haemorrhage as described by Burg *et al.* (1953) in infection with *Protostrongyloides cervi*.

(iii) *Transportation of viruses into the central nervous system*

It is evident from the work of Shope (1941, 1943*a*, *b*) and Syverton, McCoy & Koomen (1947) that nematode larvae may carry viruses from one host to another. It is therefore likely that nematode larvae entering the central nervous system may facilitate the invasion of the central nervous system by viruses present in other parts of the body. This possibility in relation to their case of poliomyelitis was considered unlikely by Beautyman & Woolf (1951). Nevertheless, Martini (1937) associated poliomyelitis with ascaris infection, and Tykać (1947) suggested that *Ascaris suum* transmits a virus causing infectious paralysis of pigs, having observed a correlation between the incidence of ascaris infection and posterior paralysis. Innes & Shoho (1952) have speculated upon the possibility that the larvae of *Setaria digitata* may transmit the virus of Japanese B encephalitis.

† Obtained by measurement of fig. 3 (Widakowich, 1905).

CONCLUSIONS AND SUMMARY

A wide variety of nematode species have been observed to invade the central nervous system. They may be located in the meningeal spaces or may penetrate into the tissues of the brain and spinal cord.

The pathological changes resulting from invasion of the central nervous system are influenced by the route of entry, the size and the mobility of the parasite. They may be diffuse or focal and may include haemorrhage, degenerative changes, cellular infiltration and glial proliferation. Such changes may or may not be observed in close association with the parasite.

Symptoms indicating involvement of the central nervous system have long been associated with nematode infections outside the central nervous system. The pathogenesis of these symptoms is obscure, but they may possibly be of allergic origin.

The direct pathological effects on the central nervous system are mainly the result of trauma and are directly proportional to the size and activity of the parasite. The possibility that nematodes may transport viruses into the central nervous system is briefly discussed.

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ON THE INVASION OF THE CENTRAL NERVOUS SYSTEM BY NEMATODES

II. INVASION OF THE NERVOUS SYSTEM IN ASCARIASIS

By J. F. A. SPRENT

University of Queensland Veterinary School, Brisbane, Queensland

(With Plates II and III and 5 Figures in the Text)

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I. INTRODUCTION

A review of the literature on nematodes in the nervous system (Sprent, 1954) has shown that several species of nematode may enter the central nervous system. The following paper records some observations on the distribution, mode of entry, development and pathological significance of the larvae of various ascaridid species in the central nervous system.

II. METHODS

Mice, 5–6 weeks old, were infected with embryonated eggs of various ascaridid species by methods previously described (Sprent, 1952). The brains of the mice were macerated in the Waring blendor and digested at 37° C. with 50 ml. physiological saline and 0·025 g. trypsin for 3 hr. at pH 7. For histological study the brain was fixed in Helly's fluid and stained with haemotoxylin and eosin. Other animals were infected by mixing the eggs with the normal food. Their brains and other tissues were digested with pepsin (1 g./100 g. brain) at pH 1 for 12–18 hr. The sediment was resuspended in 250 ml. physiological saline and left to stand for ½ hr. This was repeated several times until the supernatant was clear.

III. EXPERIMENTAL PROCEDURE AND RESULTS

(a) *Occurrence of ascaris larvae in brain of mice*

The relative distribution of the larvae of several ascaridid species was investigated by infection of four series of mice. In the first series there were nine groups of thirteen mice; each group was infected with 2500 eggs of one of the following species: *Ascaris lumbricoides*, *A. suum*, *A. columnaris* (from skunk), *A. devosi*, *Toxocara canis*, *T. mystax*, *Toxascaris leonina*, *T. transfuga* and *Parascaris equorum*. One mouse from each group was killed on each consecutive day up to 13 days after infection. In none of the mice infected with *A. lumbricoides*, *A. suum*, and *P. equorum* were larvae found in the brain or carcass. In *T. leonina* infections there were larvae in the carcass but none in the brain; in the other infections larvae were recovered both from the brain and carcass. The most striking difference among the various species was the relative abundance of the larvae of *T. canis* in the brain; Table 1 shows the average number of larvae collected from the brain and carcasses of the mice in this series. It is evident that the total number of larvae and also the proportion of migrating larvae which reach the brain is greater in *T. canis* infections than with other species.

Table 1. *Average and relative numbers of larvae found in brains and carcasses of mice infected with ascaris larvae (first series—1–13 days after infection)*

	Average number of larvae*		Brain/carcass
	Brain	Carcass	% ratio
A. columnaris (skunk)	0·23	550	0·04
A. devosi	1·23	1364	0·09
T. canis	90·15	283	31·85
T. mystax	0·84	117	0·72
T. transfuga	1·97	76	2·60
T. leonina	0·0	4	0·0

* Each figure represents the average number of larvae in the brain and carcass of thirteen mice killed on 13 consecutive days.

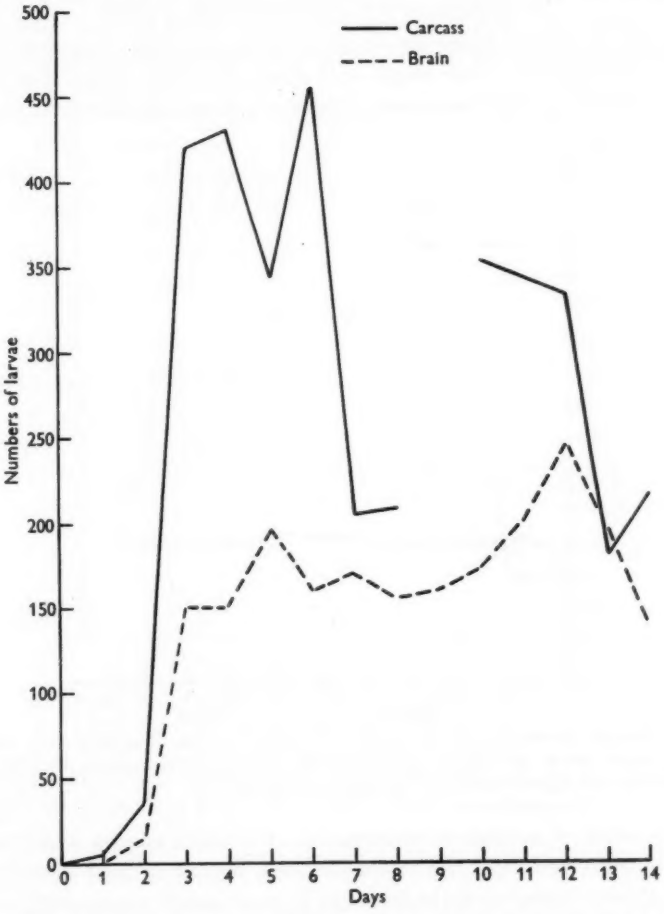
Table 2. *Average numbers of larvae occurring in the brain of mice following experimental infection with various species of ascaris*

	A. columnaris		*A. devosi*		*T. transfuga*		*T. mystax*		*T. canis*	
Duration of infection	No. of mice killed	Av. no. of larvae	No. of mice killed	Av. no. of larvae	No. of mice killed	Av. no. of larvae	No. of mice killed	Av. no. of larvae	No. of mice killed	Av. no. of larvae
2nd series, 1–14 days,	—	—	18	0·2	21	0·8	14	0·2	10†	118
3rd series, 3–24 weeks	7†	2·6	13	0*	10	1·0	6†	1·3	7†	82

* Except for one disintegrated larva at 6 months after infection.
† Some mice died during the experiment and were discarded.

A second series of mice containing four groups was infected with 2000–3000 eggs of *A. devosi*, *T. transfuga*, *T. Mystax* and *T. canis* respectively. The mice were killed on consecutive days after infection up to 14 days. The number of mice killed and the average number of larvae in the brain of each group is shown in Table 2.

A third series of mice containing five groups was infected with 2000–3000 eggs of *A. columnaris*, *A. devosi*, *T. transfuga*, *T. mystax* and *T. canis* respectively. The mice in each group were killed at 3 and 4 weeks, and thence at monthly intervals up to 6 months after infection. The number of mice killed in each group and the average number of larvae collected from the brain are shown in Table 2.



Text-fig. 1. Number of *T. canis* larvae in brain and carcass of mice 1–14 days after infection.

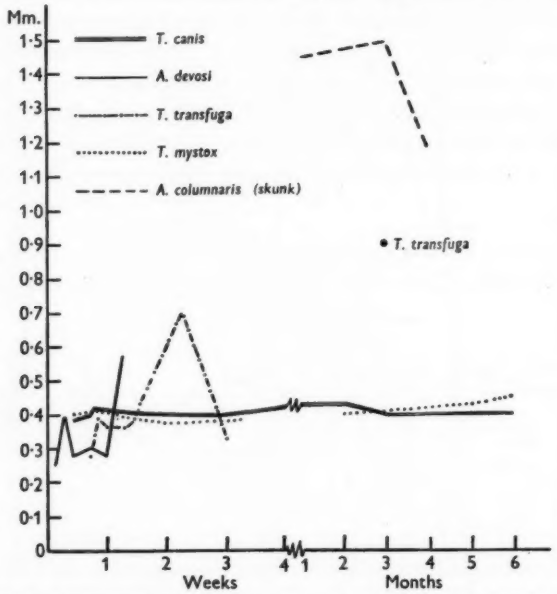
A fourth series of mice was infected with 5000 eggs of *T. canis*; one mouse was killed each day after infection. The number of larvae in the brain and carcass of these mice is shown in Text-fig. 1, which shows that penetration of the brain mostly occurs between 2 and 3 days after infection.

In each experiment the relatively greater abundance of the larvae of *T. canis* in the brain was apparent.

The larvae of *A. columnaris*, *T. canis* and *T. mystax* remained alive in the brain up to 6 months after infection; the larvae of *T. transfuga* were not found later than 12 weeks after infection, and the larvae of *A. devosi* were not observed in the brain later than 10 days after infection except for one mouse killed at 6 months in which a partially disintegrated larva was found.

(*b*) *Development of larvae in brain of mice*

The larvae recovered from the brain of experimentally infected mice were measured with regard to their length and width (Text-figs. 2, 3). For the larvae of *T. canis*, occurring in the brain more frequently than other larvae, it was possible

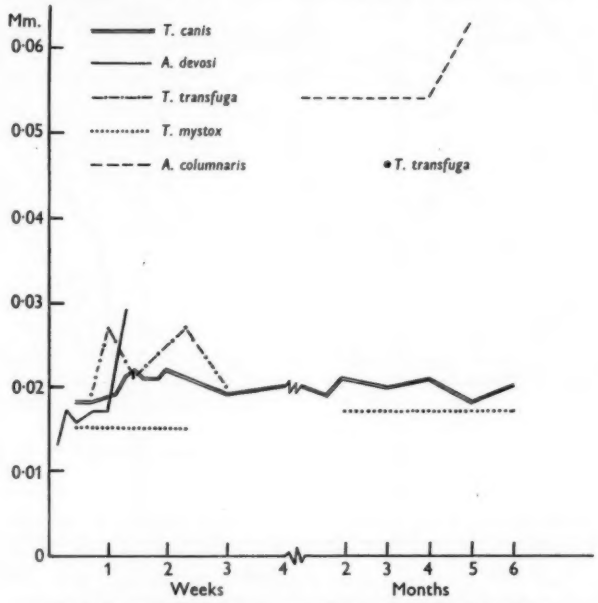


Text-fig. 2. Length measurements of larvae of various species of ascaris from the brain of mice (for *T. canis* larvae each figure represents the average measurement from three larvae, in other species each figure represents a single measurement).

to obtain a series of average measurements. For other species which were only occasionally recovered from the brain, the data regarding their growth was less complete. It was evident that the larvae of *T. canis* and *T. mystax* did not increase appreciably in size during their sojourn in the brain; a few larvae of *T. transfuga* and one larva of *A. devosi* showed evidence of growth; the larvae of *A. columnaris* showed that considerable growth had occurred in the brain. Larvae of the different species showed constant and characteristic differences in size and shape, but no feature could be distinguished that explained their relative distribution to the brain. The anterior portion of larvae of five species collected 4 days after infection are shown in Pl. XIII, figs. 1–5.

The width of the larvae being possibly an important factor in determining their distribution to the brain, and in so far as insufficient larvae were available from the

brain, a series of average width measurements of larvae from other tissues were made at daily intervals after infection up to 14 days and also at 21 and 28 days after infection. Owing to the variations in migratory behaviour of the different larvae, they were obtained from various tissues as follows: the larvae of *T. canis*, *T. mystax*, *A. devosi* and *A. columnaris* were obtained from the carcass for all measurements, those of *T. leonina* and *T. transfuga* were obtained from the wall of the intestine for the first 7 days and thereafter from the carcass, those of *A. lumbricoides* and *A. suum* were measured from the liver for the first 4 days and



Text-fig. 3. Width measurements of larvae of various species of ascaris as in Fig. 2.

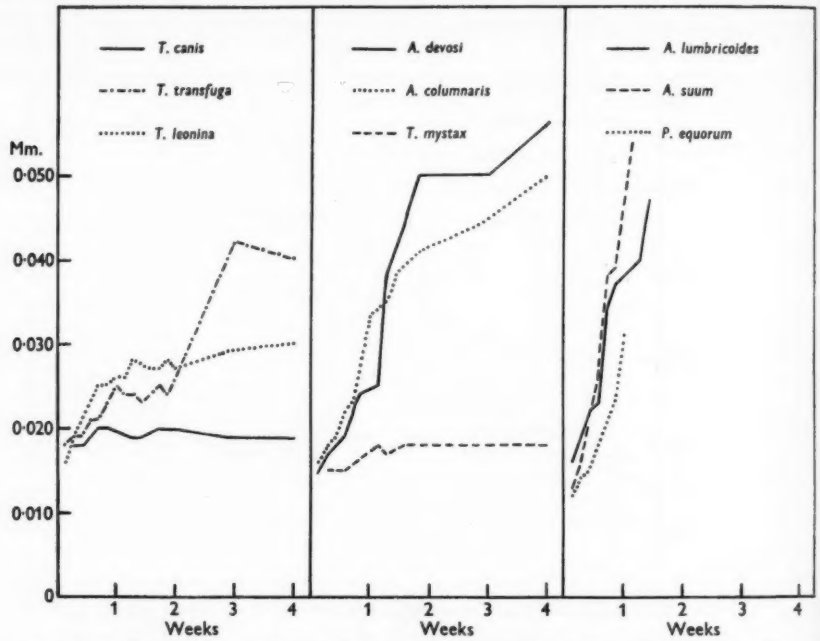
thereafter from the lungs, the larvae of *P. equorum* were measured from the liver for 7 days and thereafter from the lungs. These measurements are shown in Text-fig. 4.

(*c*) *Nervous symptoms observed in infected mice*

In mice infected for periods between 3 weeks and 6 months, symptoms were observed in twelve out of thirty-two infections with *A. columnaris*; in two out of twenty infections with *A. devosi*; in one out of twenty-three infections with *T. canis*. Symptoms did not commence before the third week of infection, and occasionally did not appear until several months after infection. The symptoms observed resembled those described by Tiner (1953*a*, *b*), though they varied in different mice and consisted of various forms of locomotor inco-ordination, such as rotation of the head and trunk (Pl. II, fig. 6), rolling and circling. The mice appeared to feed normally and often survived for several weeks while manifesting these symptoms. Complete recovery was not observed, although in some cases the

symptoms were ameliorated or changed in character. No symptoms indicating disease of the nervous system were observed in infections with the other species of ascaris investigated, or in any uninfected mice.

Some mice infected with various species died in the early stages of infection, but in these cases nervous symptoms were not apparent, although cerebral haemorrhages were often present; death was most likely a result of pulmonary damage.

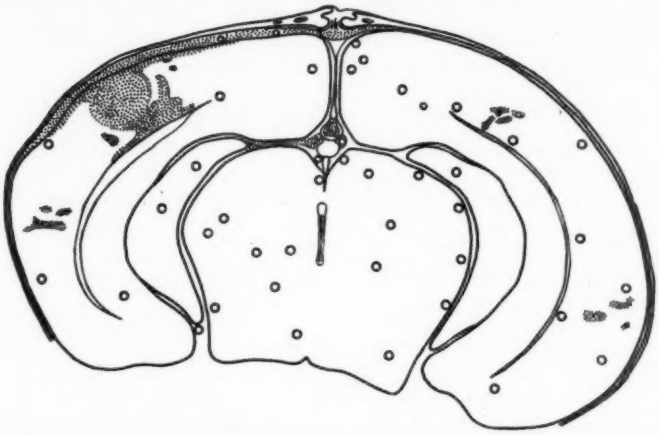


Text-fig. 4. Width measurements of larvae of various species of ascaris from the tissues of mice (each figure represents the average measurement from ten larvae).

(*d*) *Macroscopic lesions in brain of infected mice*

Commencing usually on the second or third day of infection in mice infected with the larvae of *T. canis* and *T. mystax*, haemorrhages appeared on the surface of the brain (Pl. II, fig. 7). In infections with *A. columnaris*, *A. devosi* and *T. transfuga* they usually appeared 3–4 days after infection. The haemorrhages were most conspicuous on the dorsal surface of the cerebral hemispheres; they gradually disappeared by the end of the second week. On microscopic examination the haemorrhages were found to originate from arteries at the point where they dip into the surface of the brain. Thin slices containing the haemorrhages were cut from the surface of the brain and examined microscopically. The diameter of the blood vessel at the centre was measured and found to vary between 0·009 and 0·038 mm. The appearance and distribution of these haemorrhages indicated that the larvae penetrate out of the arteries near the surface of the brain, so that the blood flows out of the vessel into the perivascular space and spreads into the subarachnoid space so as to form a characteristic circular haemorrhage which

sometimes measured 6 mm. in diameter. Later, it appeared that the fluid ascending in the perivascular space displaced the blood, thus forming a clear area in the centre of the haemorrhage (Pl. II, fig. 8). The central clear area increased considerably, and at 8 days the haemorrhage consisted of an irregular ring of agglutinated erythrocytes and brown granular clumps (Pl. II, fig. 9).



Text-fig. 5. Diagram showing distribution of larvae and haemorrhages in 150 serial sections (0·007 mm.) of the brain of a mouse infected 3 days previously with *T. canis* (dotted areas denote haemorrhage, circles denote larvae).

(*e*) *Microscopic lesions in brain of mice infected with larvae of* Toxocara canis

Sections of the brain of mice infected with larvae of *T. canis* were examined for the presence of larvae and for evidence of damage to nervous tissue. Three mice were killed each day for the first 9 days and at 14, 21, 28 and 35 days after infection. A study of the brain sections of these mice showed that the larvae had begun to invade the brain 2 days after infection, at which time a few free larvae were observed in the brain tissues; haemorrhages were evident mostly near the dorsal surface of the brain. At 3 days the number of larvae increased markedly; they were lying freely in the brain tissue and some of them were associated with areas of haemorrhage (Pl. II, fig. 10). These haemorrhagic areas were of irregular shape and in serial sections could be traced for relatively long distances, indicating that the larvae had begun to migrate through the brain tissues. At 4 days larger haemorrhages were observed, especially in the subdural and subarachnoid spaces and in the dorsal regions of the cerebellum and cerebral hemispheres. Serial sections through the brain of mice killed at 2, 3 and 4 days after infection (Pl. II, fig. 11; Text-fig. 5) suggested that most of the larvae penetrate into the dorsal region and thence spread to all parts of the brain. On subsequent days after infection there was little change in the microscopic picture, except for the absence of surface bleeding; larvae were observed lying freely in various parts of the brain and the larvae appeared as though they were in motion at the time of fixation (Pl. III, fig. 12).

Only one mouse infected with *T. canis* larvae was observed to show nervous symptoms. This mouse was kept for 4 months without showing any symptoms, but at this time began to manifest unsteady and staggering gait. It was unable to walk in the normal position and kept falling over on to its left side, the left legs slipping under the body. After 2 weeks its condition had not improved; it could no longer progress in a forward direction, but would sit up and fall over backwards. It was killed and serial sections were made of the brain. The distribution of the larvae in the brain of this mouse is shown in Table 3.

Table 3. *Distribution of ascaris larvae in brain sections of experimentally infected mice*

Mouse no.	1328	1344*	1345*	1346	1347
Infection	*T. canis*	*A. columnaris*	*A. columnaris*	*A. columnaris*	*A. columnaris*
Duration (days) ...	112	26	28	25	37
Cerebral hemispheres	57	3	2	3	6
Ventricles and chorioidal tissue	15	0	0	0	0
Hippocampus	12	1	1	0	0
Midbrain	17	2	1	0	0
Pons	8	0	0	1	0
Cerebellum	25	2	0	1	0
Medulla	5	0	1	0	1
Olfactory lobes	2	0	0	0	0
Cranial nerves	3	0	0	0	0
Meninges	15	0	0	0	0

* Only one side of the brain was available for sections.

The larvae were scattered throughout all parts of the brain; their appearance usually indicated that they had been active at the time of fixation and that they passed frequently from one part of the brain to another, migrating between the brain tissues and the meningeal and ventricular spaces. There was no evidence of meningeal or perivascular cellular infiltration, or of granuloma formation, in the brain tissues of these mice.

(*f*) *Microscopic lesions in the brain of mice infected with larvae of* Ascaris columnaris

The eggs of this parasite being no longer available to the writer, histological observations were limited to a series of nine mice. These mice were infected with 2000 eggs and were kept under observation for nervous symptoms. Mouse 1343 died at 19 days after infection without showing symptoms; owing to putrefaction no tissues were fixed. Mouse 1344 became paralysed on the right side of the body at 26 days after infection, its left limbs were stretched outwards and backwards. It was killed and serial sections of the left side of the brain and of the whole spinal cord showed the presence of eight larvae in the brain. Their distribution is shown in Table 3; some of the larvae were tightly coiled (Pl. III, fig. 13), while others were extended (Pl. III, fig. 14). Sometimes the larvae were associated with a lesion consisting of an amorphous mass infiltrated with lymphoid and eosinophilic elements (Pl. III, fig. 15); in other instances the larvae were free. In the former instances, the body of the larva was often coated with amorphous pink-

staining material, although the head was usually free. There was evidence of considerable damage to the cerebellum; in one gyrus the normal cells had been entirely destroyed and replaced by a mass of degenerated and infiltrated tissue interlaced by fusiform spaces (Pl. III, fig. 16). There were other severe lesions in the midbrain and cerebral hemisphere.

Mouse 1345 died on the same day but without showing symptoms. Serial sections of half the brain showed the presence of five larvae distributed as shown in Table 3. There was no damage to the cerebullum, but except for the larva in the hippocampus, each larva was associated with a distinct lesion.

Mouse 1346 started nervous symptoms 25 days after infection; the head was inclined with the right side towards the ground, the left limbs were stretched backwards and it circled clockwise. It was killed and serial sections were made of the whole brain and spinal cord; the distribution of larvae in the brain is shown in Table 3; no larvae were observed in the spinal cord. Four of the larvae in the brain were in an extended condition and were not associated with lesions (Pl. III, fig. 17). However, there was an extensive amount of damage in the region of the pons and cerebellar peduncles.

Mouse 1347 commenced nervous symptoms 37 days after infection; it manifested twitching of the head, backward movement and somersaulting. It died 5 days later and serial sections were made of the brain and spinal cord. The distribution of larvae is shown in Table 3.

Mouse 1348 was killed 4 months after infection, it had shown no nervous symptoms. Fresh sections about 1 mm. thick were made of its brain and spinal cord; a larva was found on the inner side of the left cerebral hemisphere near the central longitudinal fissure.

Mouse 1361 showed no symptoms until 6 months after infection when it began to roll against the side of the cage. As long as it was pressed against the side it would behave normally, but if it was turned in the opposite direction it would roll over to the other side of the cage; it rolled consistently to the left. This continued for 2 days, after which time the mouse gradually improved its locomotion, until a week later it could remain erect, although it could only progress in a clockwise circle. The mouse was killed and serial sections of the brain were made; no larvae were found but there was evidence of considerable damage to the brain in the region of the hippocampus, pons and medulla on the left side (Pl. III, fig. 18). The two remaining mice were killed 6 months after infection; they had shown no nervous symptoms and no larvae were recovered from the brains.

The lesions associated with the larvae in the brains of these mice were remarkable in their variation. In some instances the larvae were lying freely, either coiled or extended in the brain substance with no signs of damage or reaction, indicating that they were active at the time of fixation; in other instances there was evidence of cellular infiltration and tissue damage near or around the larva. Sometimes, however, there was severe damage to the brain tissue, but no larvae could be found in the vicinity.

The most striking differences observed between the microscopic picture of *T. canis* and *A. columnaris* infection of the brain was: (1) the extent of tissue

disturbance, (2) the size of the larvae (cf. Text-figs. 2 and 3). Microscopic changes following infection with *T. mystax* and *A. devosi* resembled those seen in *T. canis* infections except that larvae were very rarely observed.

(*g*) *Ascaris larvae in brain and spinal cord of pigs*

Five pigs suffering from periodic attacks of posterior paralysis were killed; the brain and spinal cord were removed and separately digested with pepsin. In the brain of one animal two ascaris larvae were recovered; these larvae were compared with larvae of several ascaris species and it was concluded that they were *A. suum*. Their measurements, compared with a larva of *A. suum* of approximately the same size, from the carcass of a mouse, are shown in Table 4.

Table 4. *Measurements of two ascaris larvae from pig brain compared with larva of* Ascaris suum *from carcass of mouse*

Larva	1	2	*A. suum*
Length	0·472	0·525	0·462
Width	0·023	0·027	0·025
Tail	0·033	0·033	0·036
Anterior end to excretory pore	—	0·067	0·077
Anterior end to end of oesophagus	0·142	0·136	0·120

A 6-month-old pig was infected with 100,000 eggs of *A. suum*. Ten days later it was killed and the intestine, liver, lungs, kidneys and brain were searched for larvae. Many larvae were recovered from the lungs and intestine but none was found in the brain.

(*h*) *Ascaris larvae in brain of dogs and cats*

The brains of twenty dogs and ten cats, destroyed at the Veterinary Clinic, were digested and examined for ascaris larvae; six of the dogs had died following nervous symptoms. In none of these animals were larvae recovered from the brain. None of six new-born puppies, though they all harboured larvae of *T. canis* in the lung, contained any larvae in the brain. Six newly born kittens contained no larvae in the brain, liver, lungs and kidneys.

Twelve dogs were given 3000–5000 eggs of *T. canis* in the food. They were killed at various times after infection (3–23 days) and the lungs and brain were digested and searched for larvae. Two dogs showed no larvae in any organ, eight dogs showed larvae in the lungs but none in the brain, and two dogs showed larvae both in the lungs and brain. Two larvae of *T. canis* were recovered from the brain of each of these dogs; they measured 0·370–0·400 mm. in length and 0·017–0·019 mm. in width. These two dogs were killed 8 days after infection.

(*i*) *Ascaris larvae in brain of horses*

The brain of a foal with a very heavy natural infection of the intestine with *P. equorum* revealed no larvae. The brain of a second foal harbouring many 4th-stage *P. equorum* larvae in the intestine, an indication of recent infection, showed no larvae. A third foal was given four weekly infections of 60,000 embryonated

eggs of *P. equorum*. Four days after the last infection the foal was killed. The liver, spleen, lungs and intestine revealed abundant larvae, but none was found to have reached the brain.

(*j*) T. canis *larvae in brain of rodents*

A series of animals as shown in Table 5 were infected with 5000 eggs of *T. canis*. Each pair of animals consisted of a young and an old animal. They were killed 7 days after infection and the larvae in the brain were counted and measured. Two rabbits were also infected, but no larvae were found in the brain.

Table 5. *Incidence and size (mm.) of* Toxocara canis *larvae in brain of rodents (7 days after infection)*

Animal	Weight	No. of larvae in brain	Average width (mm.)	Average length (mm.)
Mouse	31·8	60	0·021	0·414
Mouse	36·8	50		
Rat	91·1	20	0·022	0·424
Rat	286·0	3		
Guinea-pig	234·0	5	0·021	0·401
Guinea-pig	585·0	4		

The results are shown in Table 5; it appeared that the mice were more liable to invasion of the brain than other rodents. There was no significant change in the size of the larvae in the different hosts.

IV. DISCUSSION

The foregoing experiments have demonstrated conclusively that the larvae of *T. canis* reach the brain of mice in relatively large numbers and remain there for several months. The occurrence of cerebral haemorrhages in the early stages of infection suggests that the larvae gain access by the blood stream as was indicated by the work of Fülleborn (1921), Yokogawa (1923) and Yamaguchi (1925). The size of the cerebral blood vessels in relation to the width of the larvae and the distribution of the larvae in sections of the brain, indicate that the blood vessels are ruptured by the larvae at or near the surface of the brain. If it be assumed that ascaris larvae are distributed via the arterial blood stream, the absence from the brain of mice of the larvae of *A. lumbricoides*, *A. suum* and *P. equorum* can be explained as due to their predominantly tracheal migration on reaching the lungs (Sprent, 1952). Before reaching the arterial blood, nematode larvae must pass through the lungs and, according to Fülleborn (1925), their behaviour in the pulmonary blood vessels is to a large extent dependent upon their size. He concluded that the pulmonary capillaries are less easily traversed than the systemic capillaries, the former having a maximum diameter of 0·01 mm. when fully distended, while the latter allow the passage of objects to up 0·02 mm. in diameter. According to his conclusions nematode larvae with a diameter greater than that of the pulmonary capillaries break through into the alveolar spaces, while smaller larvae are able to pass into the pulmonary veins. Hookworm and other strongyle larvae and the larvae of *A. suum*, *A. lumbricoides* and *P. equorum*, all of which have

a diameter greater than 0·02 mm. at the time they reach the lungs, would fall into the first category, while the circulating larvae of *Trichinella spiralis* and the microfilariae, all of which have a diameter less than 0·01 mm., would fall into the second category.

The larvae of *T. canis*, *T. mystax*, *A. devosi*, *A. columnaris* and *T. transfuga* have been shown to reach the lungs of mice at a relatively early stage of infection and to undergo a somatic migration (Sprent, 1952). On the other hand, the minimum diameter of these larvae is never less than 0·01 mm. (Text-fig. 4) hence they cannot be included in the second category, even though they may gain the arterial blood. There are, however, at least two ways in which larvae can pass into the pulmonary veins without traversing the capillaries. One is by passing through arterio-venous anastomoses; such channels with a minimum diameter of 0·025 mm. have been demonstrated in the pulmonary circulation of many animals (Sirsi & Bucher, 1953). The second is by breaking out of the capillaries and re-entering the pulmonary veins from the alveolae. This was demonstrated by Fülleborn (1925) by recovery of the larvae of *Strongyloides stercoralis* from the pulmonary veins after injection into the trachea. The larvae of *T. canis*, *T. mystax*, *A. devosi*, *A. columnaris* and *T. transfuga* all produce pulmonary haemorrhages in the early stages of infection, and in so far as most of these larvae reach the somatic tissues, it is likely that they gain the arterial circulation by breaking the capillaries and entering the pulmonary veins. These larvae, at the time they reach the lungs, have a maximum diameter of 0·015–0·02 mm., being intermediate between the two groups mentioned above, and it may be significant that it is this third group of larvae which produce characteristic haemorrhages on the brain of mice.

Observations on the brain of mice infected with larvae of *T. canis* in the present experiments indicated that the lumen of the cerebral arteries approximates the diameter of the larvae at the point at which they enter the surface of the brain. Hence it seems likely that the larvae carried in the arterial blood stream leave the vessels at the surface of the brain. This may be partly because their progress is impeded by restricted movement, and partly because they cannot turn the relatively sharp bend in the vessel's course as it dips into the brain; laceration of the vessel is possibly facilitated by the sharp lateral alae of the larvae. No larvae were observed within blood vessels in sections of the brain of mice, and, in view of the small size of the intracerebral vessels relative to the width of the larvae, it appeared unlikely that the larvae of *T. canis* normally traverse the cerebral capillaries. After the larvae have penetrated out of the artery into the meninges or chorioidal tissues, some of them probably reach the subarachnoid space or ventricles without penetrating the brain tissues, while others gain access to the brain by penetration. Sufficient material was not available to study the behaviour of the larvae of *T. mystax*, *A. columnaris*, *A. devosi* and *T. transfuga*. It was apparent, however, that these larvae entered the brain tissues less frequently than those of *T. canis*, although they also produced haemorrhages on the brain. It seems probable that *T. canis* larvae are particularly active borers and thus more frequently gain access to the brain tissues, while other larvae are swept into the cerebro-spinal fluid. It is possible that some ascaris larvae may pass through the brain capillaries into the

veins; Fülleborn (1925) concluded that this would be possible for larvae less than 0·02 mm. in diameter. Between two and three days after infection, which is the time that penetration of the brain occurs, the average diameter of the various species was found to be as follows: *T. mystax*—0·015 mm., *A. devosi*—0·017 mm., *A. columnaris*—0·0175 mm., *T. transfuga*—0·019 mm., *T. canis*—0·019 mm. It may be significant that the two species with the greatest diameter were found most abundantly in the brain. The smaller larvae may pass straight through the capillaries while the larger ones are held up in the vessels. Smaller larvae, such as microfilariae and trichina larvae, do not produce surface haemorrhages and possibly can traverse the capillaries of the central nervous system; the latter larvae may occasionally produce perivascular haemorrhages indicating that they enter the brain tissues at a deeper level than ascaris larvae.

As indicated previously (Sprent, 1954), certain nematodes, such as the 4th-stage larvae of *Stephanurus dentatus*, the larvae of *Eustrongylides ignotus*, and filarial larvae inoculated by mosquitoes, may undergo direct migrations through the tissues and may thus gain access to the cranio-vertebral canal. Study of serial sections of the brain of mice infected with the larvae of *T. canis* and *A. columnaris* indicated that these larvae move actively in the brain, penetrating directly through the tissues, as well as leaving and entering the brain from the meningeal and ventricular spaces. It thus seems possible that they may migrate in and out of the cranio-vertebral canal; they were frequently observed in nearby tissues, such as salivary glands, muscle and connective tissue.

The larvae of *T. canis* did not grow during their sojourn in the brain (Text-figs. 2 and 3) and appeared to cause relatively little damage to the tissues. In contrast, the larvae of *A. columnaris* grew considerably, and, furthermore, at 3 weeks or more after infection nervous symptoms frequently occurred in infected mice; the larvae were found in the brain and in all cases there was evidence of severe damage to the tissues. The larvae were sometimes coiled, but in all instances appeared as though active at the time of fixation. It was clearly indicated that five to six larvae of *A. columnaris* in the brain could prove fatal, while there was usually no apparent harm caused by over 100 *T. canis* larvae. As found previously by Hoeppli (1927), there was remarkably little reaction of any kind as a result of invasion of the brain by the larvae of *T. canis*; in single infections there was no evidence of perivascular infiltration, meningeal infiltration or granuloma formation. Preliminary experiments have indicated, however, that a much more vigorous cellular reaction occurs in the brain of mice which have been subjected to repeated infections with larvae of different species before infection with *T. canis*. These experiments will be reported in a later publication.

The occurrence of the larvae of *T. canis* in the brain might be of considerable medical importance. The eggs of this species are of widespread occurrence, being passed in the faeces of puppies; consequently they must frequently be swallowed, especially by children. Experimental infection of various animals indicated that these larvae may reach the brain of dogs, guinea-pigs, rats and mice. The measurements of the larva found in the brain of a child by Beautyman & Woolf (1951) are more indicative of *T. canis* than of *A. lumbricoides*. Larvae of *Toxocara* spp. can

be identified by the asymmetrical appearance of the anterior end in lateral view (see Pl. II, figs. 1, 2); also in the later stages of infection by their relatively small size. Beaver and his co-workers (1952) found a *Toxocara* larva in a granulomatous lesion in the liver of a human patient suffering from chronic eosinophilia. Smith and Beaver (1953) infected a child with eggs of *T. canis* and observed that eosinophilia developed. According to the present experiments, the pathological significance of *T. canis* larvae appears to be slight, nevertheless further investigations should be conducted in relation to the possibility that these larvae may instigate allergic reactions or carry micro-organisms into the central nervous system.

V. SUMMARY

1. Experimental infections in mice showed that the larvae of *Toxocara canis*, *T. mystax*, *Ascaris devosi*, *A. columnaris* and *Toxascaris transfuga* reached the brain of mice; the larvae of *Ascaris lumbricoides*, *A. suum*, *Parascaris equorum* and *Toxascaris leonina* were not recovered from the brain. The larvae of *T. canis*, *T. mystax*, *T. transfuga* and *A. columnaris* remained in the brain of mice for several months.

2. Larvae reaching the brain produced characteristic haemorrhages on the surface of the cerebral hemispheres in the early stages of infection. It was concluded that the larvae reach the brain via the arterial blood stream, leave the arteries at the point where their diameter approximates that of the larvae, i.e. mostly on the surface of the brain, and penetrate into the brain from the subarachnoid space and chorioidal tissues.

3. The larvae of *T. canis* were found to occur in the brain of mice in relatively greater numbers than the larvae of other species, but only very rarely caused nervous symptoms. The larvae of *T. canis* and *T. mystax* showed no growth in the brain.

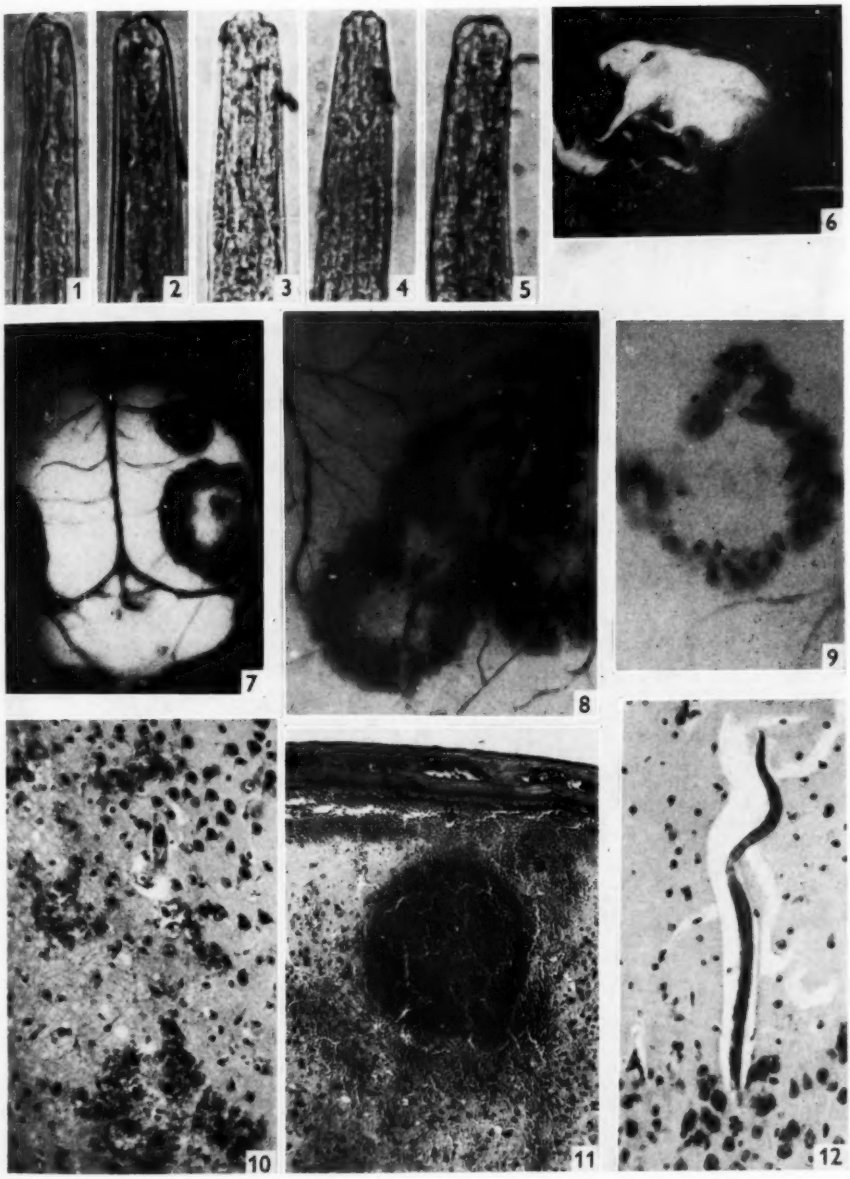
4. The larvae of *A. columnaris* (skunk) frequently caused nervous symptoms in mice, the effect appeared to result from traumatic damage due to the relatively large size attained by these larvae about 3 weeks after infection.

5. The brain of infected mice showed very slight changes consequent upon infection with larvae of *T. canis*. These larvae moved actively through the tissues; they incited little or no cellular reaction, but left haemorrhagic tracks. The larvae of *A. columnaris* also moved actively; when in the extended state they were usually found in normal tissue; when coiled, they were often associated with a necrotic focus infiltrated with leucocytes.

6. After experimental infections of dogs with larvae of *T. canis*, two out of twelve infected animals harboured larvae in the brain. No natural infections with these larvae were found in the brains of dogs and cats. After experimental infection, larvae of *T. canis* were found in the brain of mice, rats and guinea-pigs, but not of rabbits.

7. Larvae of *A. suum* were recovered from the cerebrum of one pig suffering from posterior paralysis, but not in an experimentally infected pig.

8. No larvae of *P. equorum* were found in the brain of foals in natural and experimental infections.



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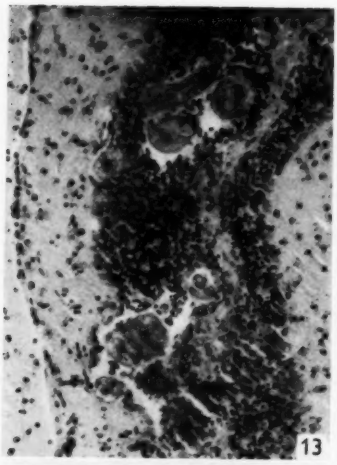
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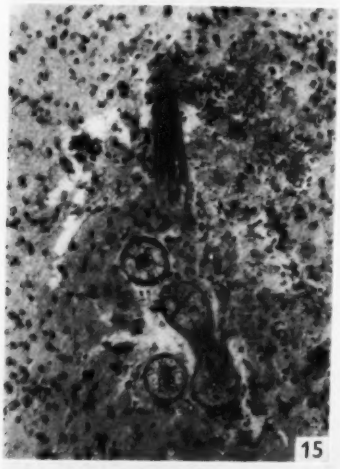
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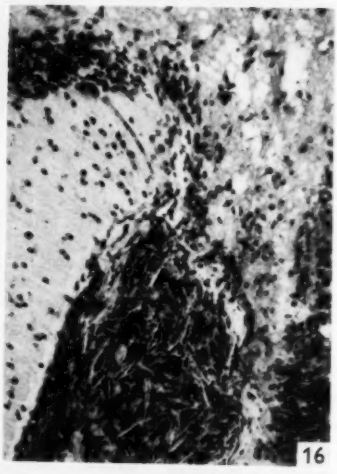
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EXPLANATION OF PLATE II (FIGS. 1–11) AND PLATE III (FIGS. 12–18)

Plate II

Fig. 1. Anterior end of larva of *T. canis*.
Fig. 2. Anterior end of larva of *T. mystax*.
Fig. 3. Anterior end of larva of *T. transfuga*.
Fig. 4. Anterior end of larva of *A. devosi*.
Fig. 5. Anterior end of larva of *A. columnaris*.
Fig. 6. Mouse 1346 showing locomotor inco-ordination (25 days after infection with larvae of *A. columnaris*).
Figs. 7–9. Haemorrhages on the dorsal surface of the brain of mice infected with larvae of *T. canis* (4–8 days after infection).
Fig. 10. Larva of *T. canis* lying near haemorrhage (3 days after infection).
Fig. 11. Haemorrhage in dorsal region of brain of mouse showing extension of haemorrhage into the meningeal spaces (3 days after infection with *T. canis*).
(Figs. 1–5, × 560; Fig. 10, × 150; Fig. 11, × 75.)

Plate III

Fig. 12. Larvae of *T. canis* in cerebrum of mouse 3 days after infection.
Figs. 13–18. Sections of mouse brain after infection with larvae of *A. columnaris* (Mice 1344–1361 see text, pp. 48–49). Fig. 13. Two coiled larvae in cerebellum. Fig. 14. An extended larva in the cerebrum. Fig. 15. Larva associated with a lesion in the cerebrum. Fig. 16. Lesion in cerebellum. Fig. 17. Free larva in cerebrum. Fig. 18. Lesion in hippocampal region.
(Figs. 12–17, × 150; Fig. 18, × 336.)

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STUDIES ON THE BOVINE FILARIID *SETARIA CERVI* (RUDOLPHI, 1819)*

By HOLMAN E. WILLIAMS, D.V.M., M.Sc.†

Department of Veterinary Science, University of Wisconsin

(With 12 Figures in the Text)

INTRODUCTION

The distribution of the peritoneal worm parasite *Setaria cervi* (Rudolphi, 1819) is almost world wide. It has been recovered from cattle, bison, buffalo, goats, from several species of deer and sheep. But aside from occasional references to its occurrence, identification and host range, little seems to be known on its life cycle and mode of transmission. In addition, there is confusion regarding its exact morphology, especially the morphology of the caudal region in the adult male. The present study was on transmission, the morphology of the microfilaria in rabbit blood and the morphology of the adult *S. cervi*.

TRANSMISSION STUDIES

Bell (1934) examined 159 Wisconsin cows and reported that 7·54% harboured microfilariae of *S. cervi* in their blood. Also, the stable fly, *Stomoxys calcitrans*, is one of the most common arthropod blood suckers among Wisconsin cattle. With these two facts in mind it was decided in the summer of 1949 to initiate transmission studies by examining these flies caught in the immediate vicinity of three University of Wisconsin cattle herds. The thorax and abdomen of over 342 of these wild *S. calcitrans* flies and the heads of fifty-four flies of the same species were thoroughly examined by dissection for the developmental larvae of *Setaria cervi* without finding a single larva. Forty-six head of cattle, which comprised two of these University herds, were then bled and the individual blood samples examined for microfilariae of *S. cervi*. Only one cow carried microfilariae in her blood. This one positive cow was examined on five occasions and the highest larval count obtained, following centrifugation, was 24·6 larvae per c.c. of whole citrated blood. The stable fly consumes about 15 mg. of blood at a meal. The chances of a fly becoming infected by feeding on this cow were considered slight. Concentration of the microfilariae by centrifugation of the infected blood was attempted. With a few exceptions the concentrates proved too viscous for the flies to take and only an occasional fly ingested a few larvae.

In order to study the possible role of the stable fly in the transmission of *S. cervi*, it was very evident that abundant numbers of microfilariae in an attractive readily ingested vehicle were necessary. Rabbits were tried. Live adult *S. cervi* worms

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† Present Address: Department of Agriculture, Trinidad and Tobago, B.W.I.

were implanted directly into the peritoneal cavities of rabbits and live microfilariae were subsequently recovered from the blood.

Adult worms were collected at a nearby packing plant from freshly slaughtered cattle. The sex ratio of 225 worms collected was 1 male to every 3·8 females. This, however, may not represent the true ratio as males were considerably more difficult to see than the females. Worms were put into rabbits 1–2 hr. after collection. Storage was at room temperature or at 37° C., the worms being immersed in physiological saline and/or physiological saline and penicillin (10,000 units in from 200 to 300 c.c. saline). Recipient rabbits were proved free of microfilariae before implants were performed. Of sixteen rabbits infected, via laparotomies, five died and eleven recovered uneventfully. Procaine penicillin in oil was usually administered after the operation at the rate of approximately 75,000 units in one injection, intramuscularly.

The earliest recovery of microfilariae from the blood of rabbits was 56 hr. following the implant. The highest level of larvae obtained was 432 per c.c. of blood in a rabbit into which 30 females and 6 males had been implanted 8 days before. In some fields under the microscope the larvae were so abundant that it was impossible to make an accurate count. The longest demonstration of larvae in the blood of a rabbit was for 45 days following the implant of the adults. Live adults were recovered from dead or sacrificed rabbits as long as 8 days following implants. On occasions, adult worms penetrated the liver, diaphragm and heart of rabbits.

It is uncertain as to whether the larvae which reached the blood were conceived in the original bovine hosts and/or in the rabbits, but in one instance a larva was recovered from an implant of female worms only.

Circulating microfilariae were sheathed and found to be as active as those in cattle. Occasionally, partly degenerated larvae were observed in wet blood smears, and one such larva was recovered from the aqueous humor of a rabbit. From all indications, the larvae survived for much longer periods in rabbits than did the adult parent worms. Similar implants into guinea-pigs proved less successful, one surviving the operation out of six. Two a-motile microfilariae were recovered from the blood of the surviving guinea-pig 15 days after implantation. An implant of 22 females and 5 males was made into a calf approximately 5 months old but no larvae were recovered from the blood. Implants into two rabbits were not accompanied by any significant changes in rectal temperatures or in differential blood determinations. Fig. 1 shows the larval levels in the blood of the two infected rabbits. Transmission studies were discontinued at this point as the winter of 1949 had commenced and stable flies were no longer available for feeding on infected rabbits.

MORPHOLOGY OF THE MICROFILARIA FROM THE BLOOD OF RABBITS

The microfilaria of *S. cervi* is long, slender and tapers to a point at the posterior extremity. It is enclosed by a structureless transparent sheath which is longer than the organism itself and allows movement of the larva forward and backward within it on occasions. Sixty-eight larvae measured from 217·4 to 261·7 μ in

length and were 6·5 μ at the widest point. The sheaths measured 265·6–297·1 μ in length and were closely applied along the wider portions of the larvae.

Wet blood smears of live larvae were fixed in a refrigerator freezing chamber for 5–10 min. This treatment usually resulted in the larvae extending themselves. Larvae were stained with Wright's stain. Fig. 2 (*A*) represents a typical stained

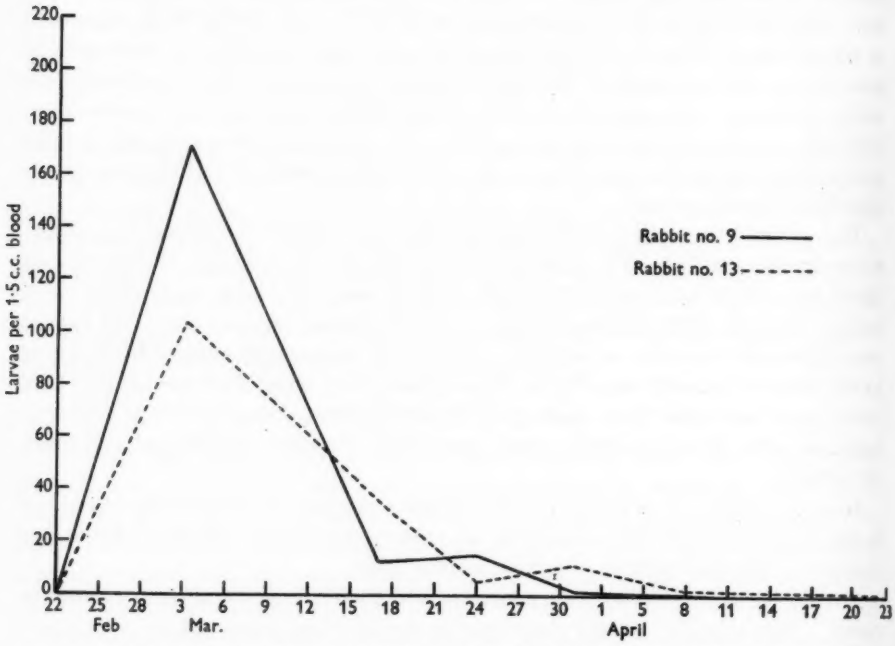

Fig. 1. Incidence of microfilariae (*Setaria cervi*) in the blood of two rabbits infected thus: rabbit no. 9, 3 males and 17 females; rabbit no. 13, 3 males and 9 females.

larva and (*B*) an unstained one. Measurements of larva (*A*), sheath omitted, were as follows: total length, 245·7 μ; width, 6·5 μ; anterior end to excretory pore, 70·2 μ; anterior end to excretory cell, 79·3 μ; anterior end to probable first genital cell, 159·2 μ; and anterior end to anal pore, 187·2 μ.

MORPHOLOGY OF THE ADULT *SETARIA CERVI*

Tables 1 and 2 present the range of measurements of five male and 10 female adult worms respectively.

The cephalic structures of both the male and female were the same. The lateral view of the head, Fig. 3, presented two distinct prominences, one dorsal and the other ventral, which were separated medially by a wide depression. These prominences when viewed dorsally or ventrally (Fig. 4), revealed that they were both indented to form twin summits. Within the medial depression arose two semi-lunar elevations leading into the esophagus. A head-on view of the cephalic region, (Fig. 5), showed the presence of two lateral papillae and four larger submedian

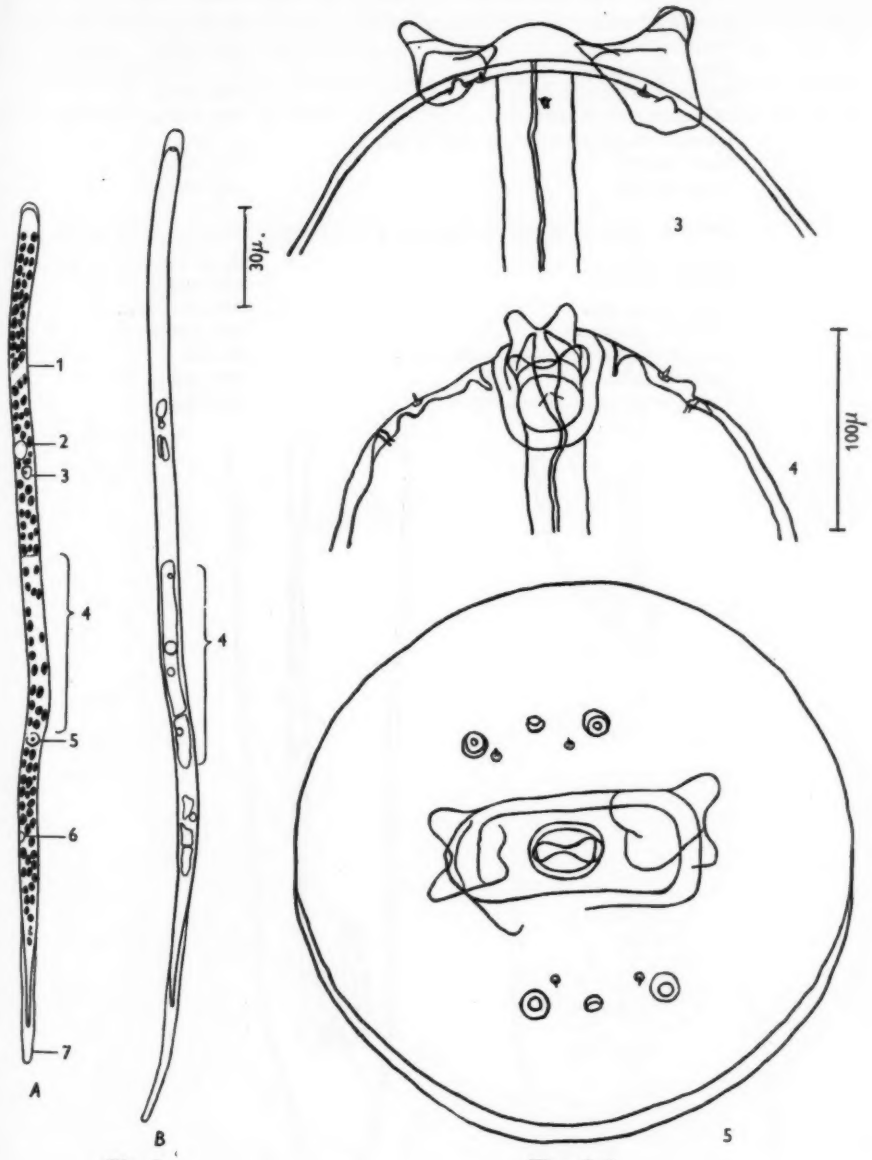

Fig. 2 Figs. 3–5

Fig. 2. *Setaria cervi*. (*A*) Stained microfilaria from the blood of a rabbit. (1) nerve ring, (2) excretory pore, (3) excretory cell, (4) central viscus, (5) probable first genital cell, (6) anal pore and (7) sheath. (*B*) Unstained microfilaria from the blood of a rabbit. (4) central viscus.

Fig. 3–5. *Setaria cervi*. Fig. 3. Anterior end, lateral view. Fig. 4. Anterior end, dorsal or ventral view. Fig. 5. Anterior end, head-on view.

Table 1. *Range of measurements of five male* Setaria cervi

Length of worm	45–51 mm.
Width of worm	326–369 μ
Total esophagus	7·7–10·3 mm.
Anterior esophagus	596–866 μ
Cloaca to end of tail	169–198 μ
Lateral caudal processes to end of tail	50–60 μ
Short spicule	117–144 μ
Long spicule	348–383 μ

Table 2. *Range of measurements of ten female* Setaria cervi

Length of worm	59–91 mm.
Width of worm	440–766 μ
Total esophagus	7·91–9·79 mm.
Anterior esophagus	667–994 μ
Lateral caudal process to end of tail	92–128 μ
Anus to end of tail	383–582 μ
Vulva to anterior extremity	468–766 μ

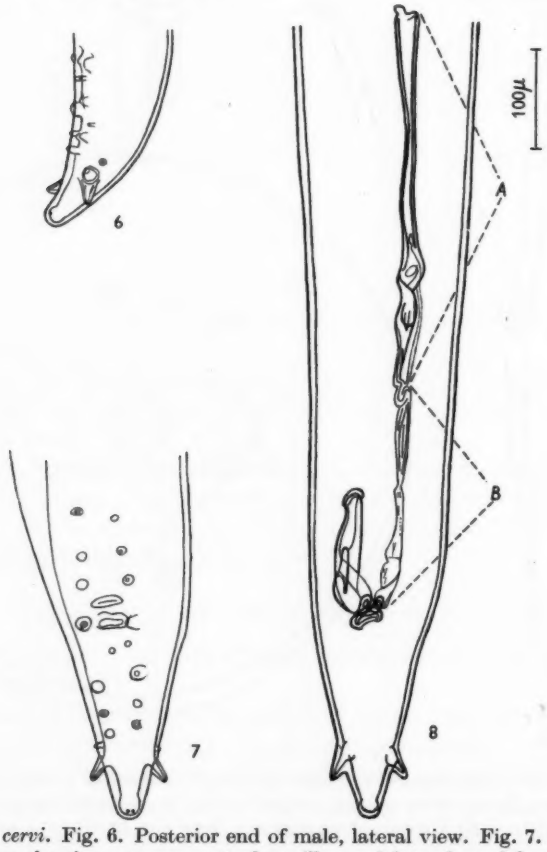

Fig. 6–8. *Setaria cervi*. Fig. 6. Posterior end of male, lateral view. Fig. 7. Posterior end of male, ventral view showing arrangement of papillae and lateral caudal processes. Fig. 8. Posterior end of male, ventral view showing cloaca, lateral caudal processes and spicules: (*A*) tubular base of left spicule; (*B*) membranous portion of left spicule.

papillae. Slightly anterior and medial to each of these submedian papillae was a minute rose thorn-like papilla. Thwaite (1927) is the only worker who reported the presence of ten head papillae.

The ventral view of the caudal portion of the male, Fig. 6, showed a cloaca, three pairs of precloacal papillae, one large pair of papillae lateral to the cloaca or

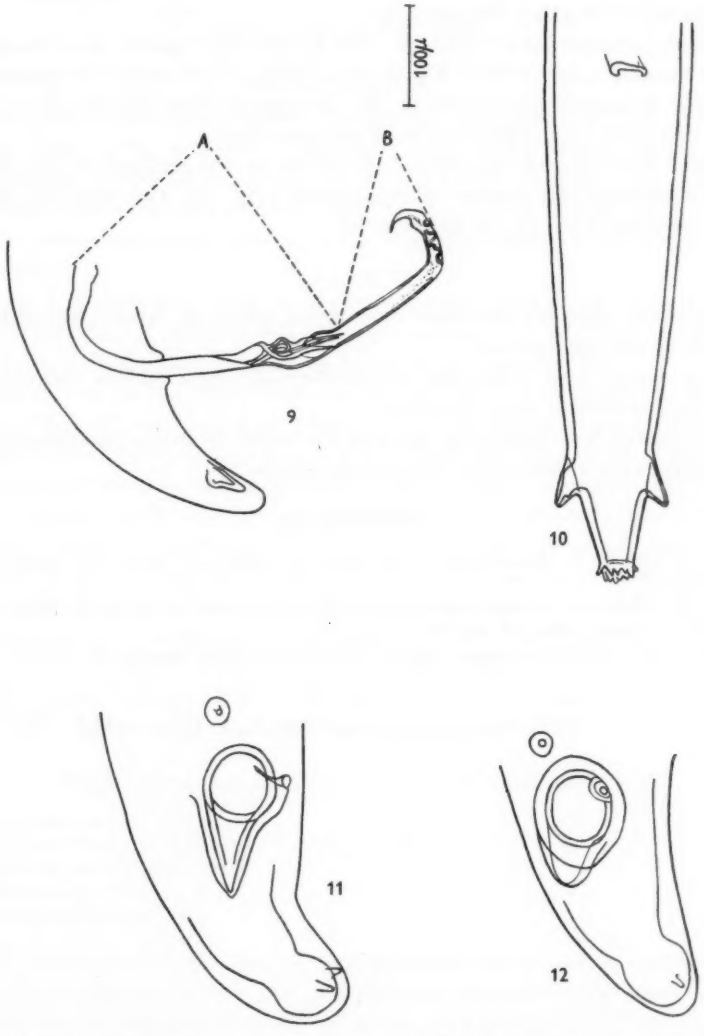

Fig. 9–12. *Setaria cervi*. Fig. 9. Posterior end, male, lateral view showing extended left spicule. Note tubular portion (*A*) and membranous portion (*B*). Fig. 10. Posterior end, female, showing anus, caudal spines and lateral processes. Fig. 11. Posterior end, male, ventrolateral view showing the most posterior post cloacal papilla, undescribed structure at the base of the lateral caudal processes and pair of projections at end of tail. Fig. 12. Same as Fig. 11 but a more direct lateral view.

adcloacal, five pairs of postcloacal papillae and immediately anterior to the cloaca was a large wide sucker-like structure, (Fig. 7). The most posterior pair of postcloacal papillae were best seen from the lateral view (Fig. 6). Proceeding posteriorly were the lateral appendages with a small duct-like structures at their anterior attachments to the body (Figs 11 & 12). A pair of small projections occurred on the end of the tail (Fig. 11). This description of the caudal region of the male closely resembles that by Stiles (1892).

The male possessed two spicules. The longer, left spicule was composed of a simple tubular anterior base, Fig. 8 (*A*) and Fig. 9 (*A*), while the posterior half was a delicate membranous portion (*B*). It appears that portion (*B*) of the left spicule may have been missed by workers heretofore.

The anus of the female opened dorsally in the caudal portion and the tip of the tail was comprised of a number of small spines (Fig. 10). The vulva of the female opened ventrally close to the anterior end.

SUMMARY

Wild *Stomoxys calcitrans* flies were dissected in search of developmental larvae of *Setaria cervi* without success.

S. cervi worms were implanted intraperitoneally into rabbits and live microfilariae were recovered from the blood.

Morphological descriptions are given of the larvae from the blood of rabbits and of the adult worms taken from slaughtered cattle.

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CONTRIBUTIONS TO THE GENUS *PARADILEPIS* HSÜ, 1935

By JUNE MAHON, D.Sc. (Neuch.) A.R.C.S.*
Institut de Zoologie, Université de Neuchâtel

(With 16 Figures in the Text)

INTRODUCTION

The genus *Paradilepis* Hsü, 1935 syn. *Meggittiella* Lopez-Neyra, 1942, is defined as follows (Hsü, 1935, p. 536): 'Dilepididae. Rostellum armed with a double crown of hooks. External segmentation little marked. Genital pores unilateral. Genital ducts pass dorsal to excretory vessels and to nerve. Testes few in number, situated to either side of the female glands. Uterus sacciform, little lobed. Parasites of birds.'

Hsü gives *P. duboisi* Hsü, 1935, as the type species of the genus: Joyeux & Baer (1935) propose that *P. duboisi* become a synonym of *P. scolecina* (Rud. 1819) Joyeux & Baer, 1935; thus *P. scolecina* becomes the type species.

The majority of the members of the genus *Paradilepis* are parasites of Pelecaniform birds, virtually confined to the cormorants. Species have been reported from birds from Africa, Europe, Australia, the Far East and from North America.

The normal techniques were employed in microscopical preparations. For the hooks, the distance from the tip of the blade to the tip of the handle is considered as the length, and that from the tip of the handle to the tip of the guard as the base. All host names cited in the text have been revised according to Peters's *Check-list of Birds of the World*, vol. I, Harvard University Press, 1931.

The species so far reported for this genus are: *P. scolecina* (Rud. 1819) Joyeux & Baer, 1935; *P. delachauxi* (Fuhrm. 1909) Joyeux & Baer, 1935; *P. kempi* (Southwell, 1921) Joyeux & Baer, 1950; *P. macracantha* Joyeux & Baer, 1935; *P. simoni* Rausch, 1949; *P. urceus* (Wedl, 1855) Joyeux & Baer, 1950.

Paradilepis scolecina (Rud., 1819), Hsü, 1935 (Figs. 1–3)

syn. *Taenia scolecina* Rud. 1819.
T. scolecina Rud., 1819 *nec* Joyeux & Baer, 1928.
Paradilepis duboisi Hsü, 1935.
P. brevis Burt, 1940.
Dilepis minima Goss, 1940.

No fresh specimens of this species were available, but prepared material from various sources was examined, and comparative measurements were made in order to gain some idea of individual variation. This material, all from *Phalacrocorax carbo* L., included the type material from Northern Europe, preparations made by Krabbe from Prussia, specimens collected by Baer from Lake Geneva, material from Roumania and preparations from the Berlin and Vienna Museums,

* Present address: Institute of Parasitology, Macdonald College, McGill University, Montreal.

collected in Europe; and lastly Hsü's type material of *Paradilepis duboisi* from *Phalacrocorax capillatus* (Temm. & Schleg.) from Peiping. The measurements obtained are given in Tables 1 and 2 below.

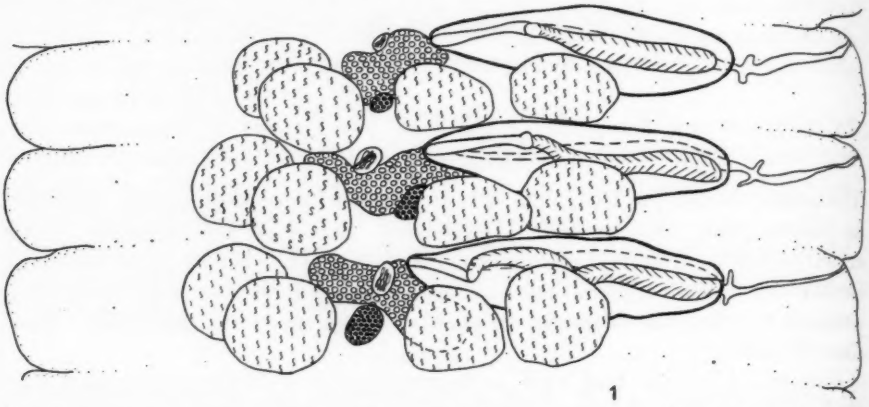


Fig. 1. *Paradilepis scolecina* (Rud. 1819) from *Phalacrocorax varius* (Gm.): dorsal view of whole mount of mature segment.

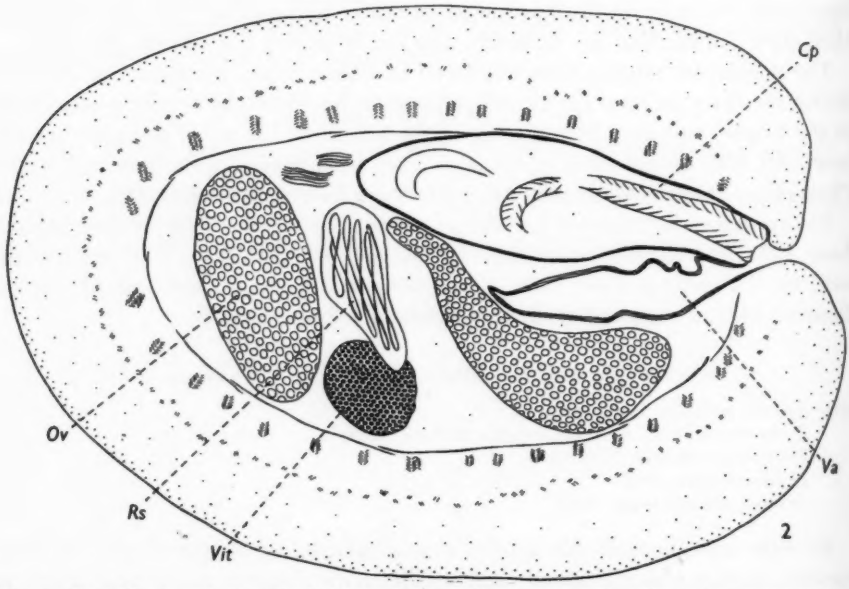


Fig. 2. *Paradilepis scolecina* (Rud. 1819) from *Phalacrocorax varius* (Gm.): tranverse section of mature segment *Cp*, cirrus pouch; *Ov*, ovary; *Rs*, seminal reservoir; *Va*, vagina; *Vit*, vitelline gland.

Rudolphi (1819) describes this species for the first time from *Phalacrocorax carbo* L. from Northern Europe, under the name of *Taenia scolecina*. Joyeux & Baer (1935) transfer *T. scolecina* to the genus *Paradilepis* (as does Hsü, 1935),

and consider *P. duboisi* to be identical with *P. scolecina*: thus *P. scolecina* becomes the type species of the genus. In a later paper, Joyeux & Baer (1950) consider *P. brevis* Burt, 1940 from *Phalacrocorax fuscicollis* Stephens, from Ceylon, to be a synonym of *Paradilepis scolecina*. The measurements given by Hsü (1935) for his *P. duboisi* and by Burt (1940) for his *P. brevis* are given in Table 2.

Goss (1940) describes a worm from Australian shags, *Phalacrocorax varius* (Gm.), *P. ater*,* *P. carbo* L. and *Haliëtor melanoleucus* (Vieill) from West Australia, and calls it *Dilepis minima*. On the examination of the co-type material received from Australia, this species is seen to be identical with *Paradilepis scolecina* (Figs. 1–3), the measurements falling well within the limits for the latter species, as may be seen from Tables 1 and 2. We propose, therefore, that *Dilepis minima* Goss, 1940 become a synonym of *Paradilepis scolecina* (Rud. 1819).

The hosts and localities from which this species has been reported are:

Host	Locality
Phalacrocorax carbo L.	Europe (Rudolphi, 1819; Joyeux & Baer, 1930)
P. carbo hanedae Kuroda	Japan (Yamaguti, 1940)
P. capillatus (Temm. & Schleg.)	China (Hsü, 1935)
P. fuscicollis Stephens	Ceylon (Burt, 1940)
P. carbo L., *P. varius* (Gm.) and *Haliëtor melanoleucus* (Vieill.)	Australia (Goss, 1940)
Haliëtor africanus (Gm.)	Africa (Joyeux & Baer, 1928, 1930)

Yamaguti (1940) obtained a single, encysted cysticercoid larva from the body cavity of *Gnathopogon elongatus caerulescens* (Sauvage) which he ascribed to the species *Paradilepis scolecina*. He gives the number of hooks as twenty, arranged in a double crown. The large hooks have a length of 105 μ and the small ones a length of 75–78 μ. The larva was found in Japan in the same locality as that in which the adults were collected.

Paradilepis delachauxi (Fuhrmann, 1909) Joyeux & Baer, 1935 (Figs. 4–6)

syn. *Oligorchis delachauxi* Fuhrmann, 1909.
Dilepis scolecina Joyeux & Baer, 1928 *nec* Rudolphi, 1819.
D. lepidocolpos Burt, 1936.

The material on which the following description is based was collected in the Belgian Congo from the cormorant *Haliëtor africanus africanus* (Gm.).

The head of the worm is completely embedded in the host's gut wall. The scolex penetrates the mucosa and submucosa, and becomes enclosed in a sac formed by the proliferation of the serous layer. The cavity of this sac is lined with a layer of acellular material, probably necrotic.

The worm measures 65–85 mm. in length; its maximum width is 2 mm. at a point about two-thirds along the total length of the worm.

* Peters (1931, vol. I, footnote, p. 90) remarks that *Carbo ater* is based on a young bird of the species *Phalacrocorax magellanicus* (Gm.) probably taken on the Falkland Islands, and that the type locality for the former 'Shark Bay, West Australia' is erroneous. It therefore seems improbable that this bird was found in Australia.

Table 1. Paradilepis scolecina (*Rudolphi*, 1819) *Joyeux & Baer*, 1935

Measurements taken from...	Krabbe, 1869	Baer, 1935 measurements Krabbe's material	Personal measurements Krabbe's material	Baer, 1935	Personal measurements Baer's material	Personal measurements of material Berlin	Vienna	Roumania
Described as...	*T. scolecina*	—	—	*P. scolecina*	—	—	—	—
Length (mm.)	7	—	7	4–7	4	3	3	6
Breadth (mm.)	0·3	—	0·37	0·22	0·33	0·23	0·18	0·32
Scolex (μ)	—	—	320, 387	230–300	238–298	223–335	170–286	285–375
Rostellum (μ)	—	—	164	140–170	149–164	134–164	88–126	134–164
Suckers (μ)	—	—	—	120–136	104–127	90–120	75–123	112–127
No. of hooks	20							
Length of large hook (μ)	93	108–115	112–115	108–117	106–113	112–129	*c.* 102	105–113
Length of small hook (μ)	64	71–85	86–89	71–85	74–79	78–92	*c.* 82	78–82
Cirrus pouch (μ)	—	—	—	92–115	102–116	78–105	62–72	94–108
Eggs (μ)	—	—	—	18–23	17–19	19–22	17–22	12–15
Testes	—	—	—	4	4	—	—	—
Host		*P. carbo* L.			*P. carbo* L.		*P. carbo* L.	
Locality		Prussia			Lake Geneva		Europe	

The scolex has a diameter of 750 μ and is provided with four, nearly spherical suckers measuring 240–290 μ in diameter. The rostellum, diameter 390 μ, is armed with a double crown of hooks (Fig. 4), twenty-two in number. The large hooks

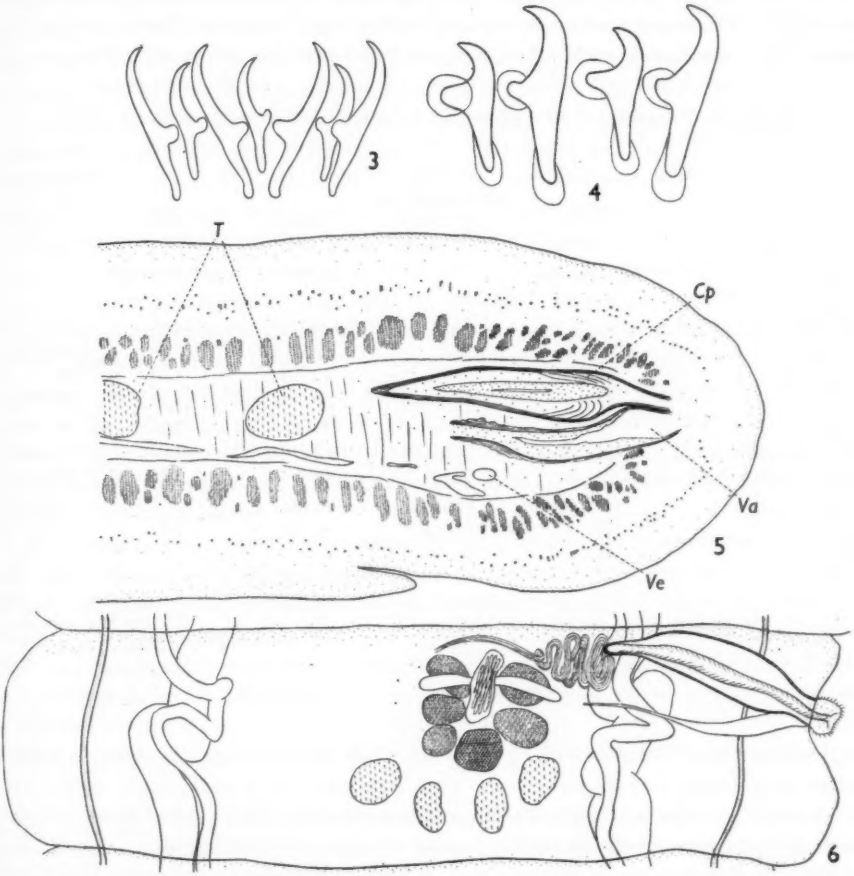


Fig. 3. *Paradilepis scolecina* (Rud. 1819) from *Phalacrocorax varius* (Gm.): rostellar hooks.

Fig. 4. *Paradilepis delachauxi* (Fuhrm. 1909) from *Haliëtor africanus africanus* (Gm.): rostellar hooks.

Fig. 5. *Paradilepis delachauxi* (Fuhrm. 1909) from *Haliëtor africanus africanus* (Gm.): transverse section of mature segment. *Cp*, cirrus pouch; *T*, testes; *Va*, vagina; *Ve*, ventral vessel.

Fig. 6. *Paradilepis delachauxi* (Fuhrm. 1909) from *Haliëtor africanus africanus* (Gm.): dorsal view of whole mount of mature segment.

measure 117–120 μ in length, and the small ones 88–91 μ. The guard and handle are provided with flattened, rounded appendages, which appear to be easily detached. The measurements given for the hooks include these disks. A comparison was made of the hook sizes of several specimens from *Phalacrocorax* sp. from Java. Two series of measurements were made, including and excluding these disks.

As may be seen from Table 3, there is a considerable individual variation in hook size.

The neck is short, and immediately behind the head, is of the same width as the scolex. On emerging into the lumen of the gut, the strobila rapidly attains a width of 1·3 mm. The segments become clearly defined only at about 10 mm. behind the head. They are much wider than long and the margins are entire. The mature

Table 2. Paradilepis scolecina (*Rudolphi*, 1819) *Joyeux & Baer*, 1935

Measurements taken from...	Hsü, 1935	Personal measurements of Hsü's material	Burt, 1940	Goss, 1940	Personal measurements of Goss' co-type material
Described as...	*P. duboisi*	—	*P. brevis*	*D. minima*	—
Length (mm.)	2·5–3·5	3	4	5·5	—
Breadth (mm.)	—	0·3	0·205–0·265	0·2	0·4
Scolex (μ)	350	350	255–340	310	296–360
Rostellum (μ)	174	149	92–102	140	96
Suckers (μ)	123	112–119	68–102	130	88–104
No. of hooks	20	—	20–22	20	20
Length of large hook (μ)	113–117	112–115	99–102	110	99–103
Length of small hook (μ)	79–81	79–81	64·6–72	100	73·5–76
Cirrus pouch (μ)	117–128	115–125	65–81	—	115–126
Eggs (μ)	18	17–19	24–27	14	—
Testes	4	4	4	—	4
Host	*P. capillatus* (Temm. & Schleg.)		*P. fuscicollis* Stephens	*P. varius* (Gm.) *P. carbo* L. *Haliëtor melanoleucos* (Vieill.)	
Locality	Peiping		Ceylon	Australia	

segment is about 10 times wider than long, while the gravid one is about 3–4 times wider than long.

There is a single set of genital organs per segment. The genital pores are unilateral, and open anteriorly on the lateral margin of the segment.

There are two pairs of well-defined, undulating excretory vessels. The dorsal canals, diameter 20–30 μ, are thick-walled. The ventral ones, 20 μ wide, are thin-walled and tend to lose their shape. The dorsal canals are wider than the ventral ones, the reverse of the usual condition. In each segment there is a single, transverse commissure, 10–14 μ in diameter.

The longitudinal musculature is composed of two bands of cortical muscles (Fig. 5). The outer, subcuticular layer consists of isolated fibres. The inner band is strongly developed and is composed of bundles of twenty to forty fibres. The medulla is clearly marked off from the cortex by a well-defined layer of transverse muscle fibres. The medulla occupies rather more than one-third of the total thickness of the segment, and is well supplied with isolated, dorso-ventral muscle fibres.

The male organs develop before the female glands, the testes being the first to

appear. The hindermost segments are almost completely filled with segmenting embryos.

The testes (Fig. 6) are four* in number, and variable in position. In certain segments one, and in others two testes are situated porally. At their maximum development, they extend almost to the dorsal and ventral limits of the medulla, and measure 110–128 μ by 85–102 μ. The vasa efferentia collect into the dorsal vas deferens, which is straight in the region of the testes, but which becomes highly convoluted as it passes porally, and enters the cirrus pouch without forming either external or internal seminal vesicles. The cirrus pouch is thick-walled and measures 272–298 μ in length and 77–93 μ at its maximum width. The cirrus is armed with numerous, backwardly directed, hooked spines, about 4·5 μ long.

The straight, thick-walled vagina opens from the genital atrium ventral to the cirrus pouch, and together with the vas deferens, passes dorsal to the excretory vessels. The vagina opens into a dorsal receptaculum seminis, a large sac-like organ filled with spermatozoa. The ovary is divided into five lobes and lies in the anterior part of the segment, posterior to the straight part of the vas deferens. The oviduct is gently coiled, and leads to the Mehlis's gland. The vitelline gland is compact, more or less spherical, and lies posterior to the ovary but somewhat in front of the testes. In the early stages of development, the ovarian and vitellarian tissues resemble each other closely, their histologies only becoming distinct in the functionally female segments.

The uterus is of the persistent, sacciform type. It appears as a small sac between the receptaculum seminis and the ovary. As it increases in size, it becomes very lobed, and filled with eggs. The uterus finally occupies the whole segment, extending laterally past the excretory canals. The lobes eventually disappear, leaving a single large sac with a continuous cavity. The ripest eggs found measure 27–34 μ in diameter. Each is provided with a granular envelope, and contains a segmenting embryo.

This species was first described by Fuhrmann (1909), from *Haliëtor africanus* (Gm.) from Egypt, but unfortunately his specimen possessed no scolex. Joyeux & Baer (1928) describe a contracted specimen from a cormorant from West Africa, possessing a head, and ascribe it to *Dilepis scolecina* (Rud., 1819). In 1935, Joyeux & Baer transfer their *D. scolecina* Joyeux & Baer, 1928 *nec* Rud. 1819 to the genus *Paradilepis* Hsü, 1935, the species in question becoming *P. delachauxi*.

Joyeux & Baer (1950) consider *Dilepis lepidocolpos* Burt, 1936 from *Haliëtor niger* (Vieill) from Ceylon, to be a synonym of *Paradilepis delachauxi*. The measurements given by Burt are shown in Table 3.

The hosts and localities so far recorded for this species are:

Host	Locality
Haliëtor africanus africanus (Gm.)	Africa (Fuhrmann, 1909; and present paper)
Haliëtor niger (Vieill.)	Ceylon (Burt, 1936)
Phalacrocorax sp.	Java (present paper)
Cormorant sp.	West Africa (Joyeux & Baer, 1928)

* Joyeux & Baer (1950) give five as the number of testes for *P. delachauxi*.

Table 3. Paradilepis delachauxi (*Fuhrmann*, 1909) *Joyeux & Baer*, 1935

Measurements taken from... Described as...	Personal *P. delachauxi*	Personal *P. delachauxi*	Fuhrmann, 1909 *O. delachauxi*	Burt, 1936 *D. lepidocolpos*
Length (mm.)	65–85	—	100	200
Breadth (mm.)	2	—	2	1·8
Scolex (μ)	750	915	500 Joyeux & Baer,	700
Rostellum (μ)	390	375	200 1928 de-	330
Suckers (μ)	240–290	300–375 × 225–300	170 scribed as	260
No. of hooks	22	20	20 *Dilepis sco-*	20
Large hook (μ)	117–120*	110–122 × 75–80* 96–110 × 59–66†	103 *lecina*	105
Small hook (μ)	88–91*	82–93 × 41–48* 68–82 × 28–37†	63	84
Cirrus pouch (μ)	272–298 × 77–93	298–335	300	¼ of breadth of segment i.e. *c.* 450
Testes	4	4	4	4 (rarely 2, 3, 5)
Eggs (μ)	27–34	Not ripe segs.	—	28
Diameter of dorsal excretory vessel	20–30	17–24	—	15
Diameter of ventral excretory vessel	20	40–50	—	60
Diameter of transverse excretory vessel	10–14	17–27	—	—
Host	*H. africanus africanus* (Gm.)	*Phalacrocorax* sp.	*H. africanus africanus* (Gm.)	*H. niger* (Vieill.)
Locality	Belgian Congo	Java	Egypt	Ceylon

* With appendages. † Without appendages.

Paradilepis kempi (Southwell, 1921) Joyeux & Baer, 1950 (Figs. 7–11)

syn. *Dilepis kempi* Southwell, 1921.
Hymenolepis kempi (Southwell, 1921) Mayhew, 1925.
Oligorchis burmanensis Johri, 1941.
Meggittiella kempi (Southwell, 1921) Lopez-Neyra, 1942.
Dilepis maxima Goss, 1940.

Fairly large numbers of this worm were present in the intestine of *Phalacrocorax* sp. from Java.

The worm measures 70–100 mm. in length. The neck region is very short and narrow, 0·26 mm. in width, and behind it the strobila gradually widens to a maximum width of 1·05 mm. at the terminal segments.

The scolex is almost spherical and has a diameter of 370 μ with the rostellum extruded (Fig. 8*a*), and 410 μ with the rostellum retracted (Fig. 8*b*). The scolex is provided with four ovoid suckers measuring 180–200 μ by 130–160 μ. The

rostellum, diameter 180–200 μ, is frequently seen everted, and is armed with twenty-eight hooks (Fig. 7) arranged in a double crown. The hooks are provided with very transparent, flattened appendages, easily overlooked in a cursory examination. Two series of measurements were made. The large hooks measure

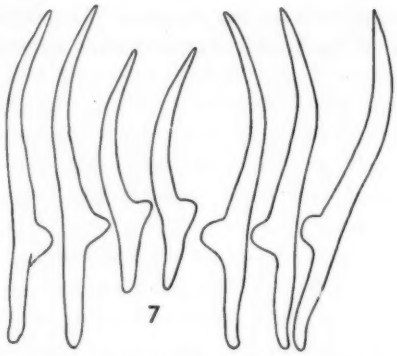
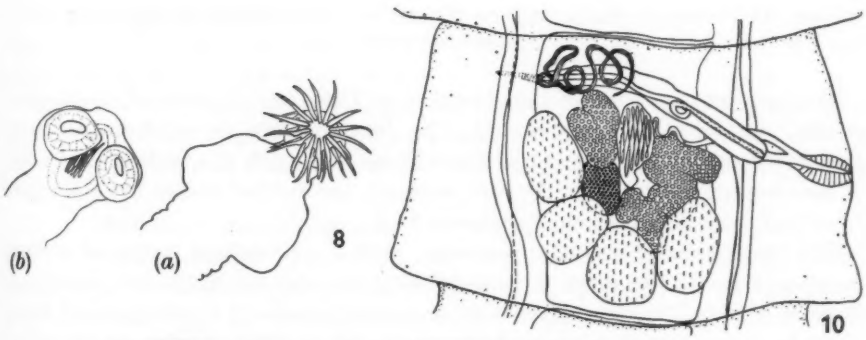


Fig. 7. *Paradilepis kempi* (Southwell, 1921) from *Phalacrocorax* sp.: rostellar hooks.



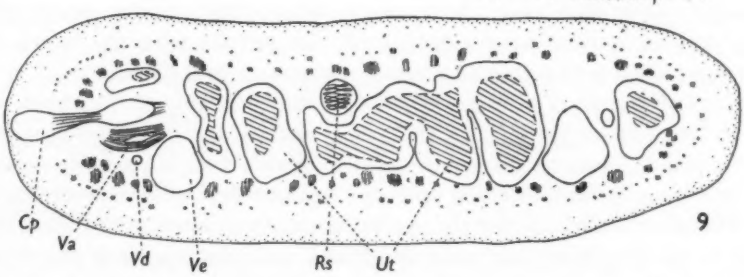


Fig. 8. *Paradilepis kempi* (Southwell, 1921) from *Phalacrocorax* sp.: scolex. (*a*) rostellum evaginated, (*b*) rostellum withdrawn.

Fig. 9. *Paradilepis kempi* (Southwell, 1921) from *Phalacrocorax* sp.: transverse section of mature segment. *Cp*, cirrus pouch; *Rs*, seminal reservoir; *Ut*, uterus; *Va*, vagina; *Vd*, dorsal vessel; *Ve*, ventral vessel.

Fig. 10. *Paradilepis kempi* (Southwell, 1921) from *Phalacrocorax* sp.: dorsal view of whole mount of mature segment.

180–190 μ including the appendages, and 173–187 μ excluding them. The small hooks have lengths of 126–133 μ and 122–126 μ respectively.

The majority of the segments are wider than long; the margins are entire. As the segments mature, they become relatively longer, and the width of the ripe segment is about three-quarters of its length.

There is one set of genital organs per segment. The genital pores are unilateral, and open in the middle of the lateral border of the segment.

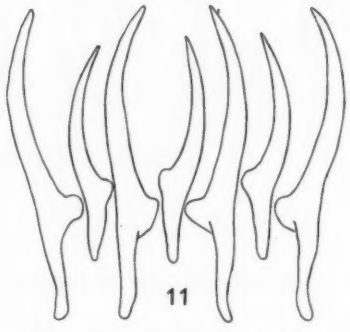


Fig. 11. *Paradilepis kempi* (Southwell, 1921) from *Haliëtor melanoleucos* (Vieill.): rostellar hooks.

The excretory system is of the normal type. There are two pairs of longitudinal canals, one dorsal and one ventral. The dorsal vessels are thick-walled, with a narrow lumen of diameter 17 μ. The ventral vessels are thin-walled and wider, of maximum diameter 50 μ. In each segment, the ventral vessels are connected by a transverse commissure, of diameter 24 μ.

The longitudinal musculature consists of two, well-defined layers of cortical muscles (Fig. 9). The outer layer is subcuticular and the fibres are grouped in bundles of four. The inner layer is strongly developed and is composed of about forty bundles each containing about twenty fibres. The circular muscles form a clearly defined, narrow band, marking off the medulla from the cortex. The medulla is provided with a limited number of dorso-ventral fibres.

There are four testes* (Fig. 10), oval in shape, and measuring 61–75 μ by 51–61 μ as seen in longitudinal section. In transverse section, the testes are seen to be dorsal in position. Their arrangement varies somewhat from segment to segment, usually one being poral and three aporal; alternatively, two are poral and two aporal. The three testes nearest the genital pore are situated in the same plane posterior to the female organs, while the fourth and most aporal is placed more anteriorly. The vas deferens is highly convoluted and leads into the cirrus pouch without forming either internal or external seminal vesicles. The cirrus pouch, measuring 223–260 μ in mature segments, and 260–298 μ in gravid ones, passes dorsally to the excretory vessels and extends along the anterior border of the

* In their key to the species of *Paradilepis*, Joyeux & Baer (1950) give three as the number of testes for *P. kempi* (*fide* Southwell).

segment almost to the aporal excretory canals. It empties into a well-developed, muscular, genital atrium.

The muscular vagina opens from the genital atrium ventral to the cirrus pouch, and passes dorsal to the excretory vessels. It leads into a well-developed receptaculum seminis. The ovary is bilobed, each lobe being sub-divided into two, three or four lobes. The yolk gland is compact and smaller than the ovarian lobes, between which it is situated in a posterior, ventral position.

The uterus appears as a simple sac, which later becomes lobed and filled with eggs, and gradually increases in size. Ultimately, the uterus fills the whole segment, extending into the cortex. The lobes disappear, and the uterus assumes the appearance of a large sac with a continuous cavity. The eggs contain an ovoid embryo, hookless, and measuring 24 by 14 μ. The shells vary considerably in shape, and appear to be deformed, but an average measurement is 35 by 25 μ.

Paradilepis kempi was first described as *Dilepis kempi* from *Haliëtor pygmaeus* (Pall.)* from Assam, by Southwell in 1921. In 1925, Mayhew transferred the species to the genus *Hymenolepis* Weinland, 1858, and in 1942 Lopez-Neyra removed it to the genus *Meggittiella* Lopez-Neyra, 1942. In 1950, Joyeux & Baer show *Meggittiella* to be a synonym of *Paradilepis* Hsü, 1935, the species in question thus becoming *Paradilepis kempi*.

As may be seen from the above description, and from Table 4, there are a number of discrepancies with Southwell's (1921), account, such as the number and size of the hooks and the number of testes.

Joyeux & Baer (1950) give *Oligorchis burmanensis* Johri, 1941 from *Phalacrocorax javanicus* (Horsfield) from Burma as a synonym of *Paradilepis kempi*. As seen from the table, Johri's measurements fall well within the limits of those given for *P. kempi*.

Goss (1940) described two cestodes from a shag, *Haliëtor melanoleucos* (Vieill.) from Western Australia. One, which she describes as *Dilepis maxima*, corresponds with *Paradilepis kempi*, and on examination of the co-type material received from Australia (Fig. 11) (measurements given in Table 4) is seen, in fact, to be identical with *Paradilepis kempi*. We propose, therefore, that *Dilepis maxima* Goss, 1940 become a synonym of *Paradilepis kempi* (Southwell, 1921) Joyeux & Baer, 1950.

The hosts and localities from which this worm has so far been reported are:

Host	Localities
Haliëtor melanoleucos (Vieill.)	Australia (Goss, 1940)
Haliëtor pygmeus (Pall.)	Assam (Southwell, 1921)
Phalacrocorax javanicus (Horsfield)	Burma (Johri, 1941)
Phalacrocorax sp.	Java (present paper)

Paradilepis macracantha Joyeux & Baer, 1935 (Fig. 12)

syn. *Dilepis delachauxi* Joyeux & Baer, 1930 *nec* Fuhrmann, 1909.

Joyeux & Baer (1935) propose this name for a worm which they described as *Dilepis delachauxi* in 1930 from *Haliëtor africanus* (Gm) from the Niger Valley. The scolex of this species is not buried in the mucosa of the host gut wall.

* Peters (1931, vol. 1) records this bird as occurring in Europe, the Middle East, Persia and Afghanistan.

Table 4. Paradilepis kempi (*Southwell*, 1921) *Joyeux & Baer*, 1950

Measurements taken from...	Personal	Southwell, 1921	Johri, 1941	Goss, 1940	Personal measurements of Goss' co-type material
Described as...	*P. kempi*	*D. kempi*	*O. burmanensis*	*D. maxima*	—
Length (mm.)	70–100	50	32–46	130	—
Breadth (mm.)	1·05	1	0·89	1·21	1·2
Scolex (μ)	370–410	400	375–400	350	480 (in glycerine)
Rostellum (μ)	180–200	160	106–120	—	160
Suckers (μ)	180–200 × 130–160	100	165–188	114	160
No. of hooks	22	20	22	20	26
Length of large hook (μ)	180–190* 173–187†	175	163–188	153	180–184
Length of small hook (μ)	126–133* 122–126†	135	109–133	108	122–126
Cirrus pouch (μ)	223–298	Small and insignificant	240–317 (–410)	300	280–320
Number of testes	4	3	—	4	4
Eggs	35 × 25	—	28–34	35 × 21	32
Host	*Phalacrocorax* sp.	*Haliëtor pygmeus* (Pall.)	*P. javanicus* (Horsfield)	*Haliëtor melanoleucos* (Vieill.)	—
Locality	Java	Assam	Burma	Australia	

* With appendages. † Without appendages.

The type material was examined. The hooks (Fig. 12) measure 425–478 μ in the length for the large ones, and 276–290 μ for the small ones, which corresponds with the dimensions given in the original description (465 by 255 μ for the large hooks and 282 by 155 μ for the small ones).

Fig. 12. *Paradilepis macracantha* Joyeux & Baer, 1935 from *H. africanus* (Gm.): rostellar hooks.

The anatomy of the strobila is similar to that of *P. delachauxi*.

This worm has not been reported since its discovery in 1930.

Paradilepis simoni Rausch, 1949

This species was described by Rausch (1949) from *Pandion haliaetus carolinensis* (Gm.) (Accipitriformes) from Wyoming, and is the first record of a species of the genus *Paradilepis* occurring in North America. It is the second worm (the other being *P. urceus* reported from Accipitriformes and Ciconiiformes) to be recorded from a bird which does not belong to the order Pelecaniformes. Rausch (1949, p. 3) remarks that, 'it is possible that *P. simoni* is an accidental parasite of the osprey and occurs naturally in

cormorants', but that 'at the present there is no reason to doubt that the osprey is the natural host of *P. simoni*'. However, Joyeux & Baer (1950, footnote, p. 94) say 'This is the second species to be reported from a fish-eating accipitrine and it appears more than likely, as suggested by Rausch, that the true host belongs either to the Pelecaniformes or to the Ardeiformes.' (Ardeiformes = Ciconiiformes *p.p.*).

For the sake of completeness, Rausch's measurements are given here. Length of worm, 50–90 mm., maximum breadth, 0·45 mm. Scolex diameter 470–596 μ and suckers diameter 180–220 μ. Rostellum 100 μ long and armed with thirty-six hooks arranged in a double crown. Large hooks 98–102 μ long and the small ones 68–72 μ long. Genital pores unilateral: testes five in number. Cirrus pouch measures 100–132 μ by 30–40 μ. Eggs diameter 27–33 μ and the embryonic hooks length 6 μ.

The only record is that in the original description.

Paradilepis urceus (Wedl, 1855) Joyeux & Baer, 1950 (Figs. 13 to 15)

syn. *Dilepis urceus* Wedl, 1855
Hymenolepis urceus (Wedl, 1855) Meggitt, 1927
H. multihamata Meggitt, 1927
Oligorchis hieraticos Johri, 1934
Meggittiella multihamata (Meggitt, 1927) Lopez-Neyra, 1942

No fresh material was available, but specimens from *Platalea leucorodia* L. (Ciconiiformes) from Looss' collection from Cairo were examined, and the measurements obtained may be seen in Table 5. Preparations from the Berlin and Vienna

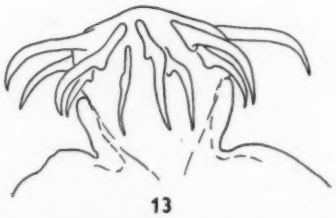


Fig. 13. *Paradilepis urceus* (Wedl, 1855) from *Platalea leucorodia* L.: scolex.

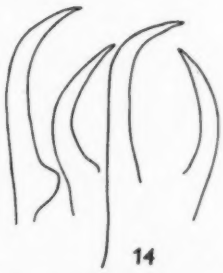


Fig. 14. *Paradilepis urceus* (Wedl, 1855) from *Platalea leucorodia* L.: rostellar hooks.

Museums were also examined, but these were found to be identical with *Paradilepis scolecina*. As may be seen from the table, the dimensions of the large hooks of *P. scolecina* and *P. urceus* (Fig. 13) fall within the same range (large hook 99–177 μ and small hook 65–85 μ for the former species; large hook 110–120 μ and small one 83–100 μ for the latter). The shapes are somewhat different, however, the tip of the hook of *P. urceus* (Fig. 14) being slightly more curved. The appearance of the entire worm is also different *P. urceus* having a short, stumpy form (Fig. 15) *P. urceus* has three to four testes, and *P. scolecina* has four.

Table 5. Paradilepis urceus (*Wedl*, 1855) *Joyeux & Baer*, 1950

Measurements taken from...	Wedl 1855	Krabbe, 1869	Personal measurements of Looss' material	Meggitt, 1927	Personal measurements of Meggitt's co-type material	Johri, 1934
Described as...	*T. urceus*	*T. urceus*	—	*H. multihamata*	—	*O. hieraticos*
Length (mm.)	4	4	2·5	2	—	6·5
Breadth (mm.)	0·33	0·3	0·37	0·3	—	0·45
Scolex (μ)	—	—	283	220–300	253	375–390
Rostellum (μ)	—	—	134	60	156	130
Suckers (μ)	—	—	112–120	—	112	110–140
No. of hooks	20	20	20	—	—	16–18
Length of large hook (μ)	110	110–111	122	115–120	—	113–190*
Length of small hook (μ)	—	73–76	89	83–95	—	83–100
Cirrus pouch (μ)	—	—	96–108	150–170 × 40	65 × 32 (mature segs. only)	65–125
Number of testes	—	—	—	3	3–4	4 (from drawing)
Eggs (μ)	48	—	23–26 × 18–19	—	—	—
Host	*Plegadis falcinella* (L.)	*Platalea* sp.	*P. leucorodia* L.	*M. migrans aegyptius* (Gm.)		*M. migrans govinda* Sykes
Locality	Austria	Europe	Egypt	Egypt		India

* Joyeux & Baer (1950 p. 93) say 'It is no doubt by mistake that Johri states the length of the large hooks as varying from 113 to 190 μ.'

This species was first described by Wedl (1855) as *Taenia urceus* from *Plegadis falcinellus* (L.) (Ciconiiformes) from Austria. Krabbe (1869) re-described the species, basing his description on specimens from *Platalea* sp.

Joyeux & Baer (1950) consider *Hymenolepis multihamata* Meggitt, 1927 from *Milvus migrans aegyptius* (Gm.) (Accipitriformes) from Egypt, to be a synonym of *P. urceus*. The co-type material of *Hymenolepis multihamata* was examined and the measurements, together with those taken from Meggitt (1927), are given in Table 5.

In the same paper Joyeux & Baer (*loc. cit.*) also make *Oligorchis hieraticos* Johri, 1934 from *Milvus migrans govinda* Sykes from India, a synonym of *Paradilepis urceus*. The dimensions given by Johri (1934) may also be seen in Table 5.

The hosts and localities reported for this worm are:

Hosts	Localities
Plegadis falcinella (L.)	Austria (Wedl, 1855)
Platalea leucorodia L.	Egypt (Joyeux & Baer, 1950)
	Europe (Krabbe, 1869)
Milvus migrans aegyptius (Gm.)	Egypt (Meggitt, 1927)
M. migrans govinda Sykes	India (Johri, 1934)

Larva: *Glossocercus cyprinodontis* Chandler, 1935 (Fig. 16)

Chandler (1935) describes a new species of larva found in the body cavity of *Cyprinodon variegatus* and *Fundulus heteroclitus* from Texas. The scolex bears twenty hooks arranged in a double crown. The large hooks measure 180 μ in length and the small ones 130 μ.

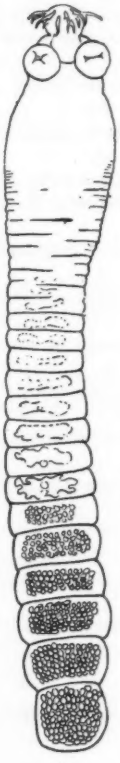


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Fig. 15. *Paradilepis urceus* (Wedl, 1855) from *Platalea leucorodia* L.: dorsal view of entire specimen.

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Fig. 16. *Glossocercus cyprinodontis* Chandler, 1935 from *Cyprinodon variegatus*: rostellar hooks (after Chandler).

Chandler remarks that his specimen does not appear to fit any described larval species and approaches the larva most closely to *Dilepis kempi*. Chandler's drawing of the hooks (1935, p. X, fig. 5) is reproduced in Fig. 16.

I would like to thank Professor J. G. Baer, Director of the Institut de Zoologie, Université de Neuchâtel, for his helpful interest in the preparation of this paper.

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(*MS. received for publication* 4. II. 1954.—Ed.)

ADDENDUM

I have been unable to take into account a paper by R. S. FREEMAN entitled *Paradilepis rugovaginosus* n.sp. (Cestoda: Dilepididae) from the Osprey, with notes on the genus *Oligorchis* Fuhrmann, 1906 that appeared in the *Journal of Parasitology*, **40**, 22–8, 5 figs., while my own paper was in the press.

THE MORPHOLOGY OF *TRYPANOSOMA STRIATI* N.SP., FROM AN INDIAN FRESH-WATER FISH*

By SYED SHAMSUDDIN QADRI, M.Sc., Ph.D.

Department of Parasitology, London School of Hygiene and Tropical Medicine†

(With 12 Figures in the Text)

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I. INTRODUCTION

The new species of *Trypanosoma* described in this paper is parasitic in the blood of *Ophicephalus* (=*Ophiocephalus*) *striatus* Bl., an air-breathing teleostean 'Serpent-fish' found extensively in the fresh waters of India. This fish is one of the important food-fishes of India, especially in areas far away from the sea, which are mainly served by inland fisheries. It is equipped with an elaborate accessory air-breathing organ and therefore survives when taken out of water and kept in a moist condition. This hardiness of the fish has made it a popular item of diet since it is available in the hottest months of the summer in a fresh condition or even alive. With the expansion of the inland fisheries of India the importance of fishes of the genus *Ophicephalus* is also on the increase.

The present study is the first of a series on the various protozoan parasites of this host and other genera of edible fresh-water fishes of India. The author has also studied in detail the life cycle of this trypanosome in culture, the results of which will be published soon.

The study of trypanosomes of fish begins with the work of Valentin (1841) who was the first to discover a trypanosome in the blood of *Salmo fario*. Since then a large number of trypanosomes have been described in fishes from many countries. The descriptions vary greatly in their scope from a mere mention or inadequate account, without proper scientific nomenclature and without any figures, to works where complete descriptions are given. Notable works on the trypanosomes of fishes are those of Danilewsky (1885), Laveran & Mesnil (1901, 1902, 1912), Léger (1904), Brumpt (1904, 1905, 1906), Petrie (1905), Robertson (1906), Neumann (1908, 1909), Minchin (1909, 1912), Minchin & Woodcock (1910), Fantham (1919), and Tanabe (1924).

As regards the trypanosomes of *Ophicephalus*, Lingard (1904) mentioned briefly two kinds of trypanosome from *O. striatus* but without nomenclature or figures.

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† Now in the Department of Zoology, College of Science, Osmania University, Hyderabad-Deccan 7, India.

Mathis & Léger (1911) and Wenyon (1908) have also reported the presence of trypanosomes in some species of *Ophicephalus* with a brief account of the parasites but without naming them and without figures.

II. MATERIAL AND METHODS

O. striatus used for this investigation were collected from lakes and rivers of Hyderabad State, India, and were brought alive by the author to London and kept in a living condition at the School of Hygiene and Tropical Medicine. In all, eighteen fish of this genus were examined. Of these, five were found to harbour in their blood the species described here. The infection was not heavy in any specimen.

For the observation of living parasites hanging-drop preparations were employed. Fresh films were examined in normal saline with methyl blue and eosin, and fixed preparations of numerous blood smears were made. Organ smears were also prepared, but they revealed no trypanosomes. Wet blood films were fixed in osmic acid vapour. Methyl alcohol, Schaudinn, Flemming and Carnoy solutions were also used as fixatives. Staining was carried out either by Leishman's or Giemsa's methods, or with Heidenhain's iron haematoxylin (alcoholic and aqueous) stain. The best results were obtained with methyl alcohol as fixative and Giemsa's stain. The blood used for making films was taken directly from the heart of the fish. The figures illustrating this paper were made with a camera lucida at a uniform magnification of 2500.

III. DESCRIPTION

In fresh films the trypanosome of *Ophicephalus* can be detected by its motility amongst the red and white blood corpuscles. Two forms, a small and a large, are seen, the small being very actively motile while the large one is stout and sluggish.

In blood preparations stained by Giemsa's method, however, the organisms are more markedly polymorphic, consisting of three forms, namely, small (Fig. 1), intermediate (Figs. 2–8) and large (Figs. 9–12), the latter being very broad and stumpy and by far the commonest. The smaller the body the denser and more stainable is the cytoplasm, so that the stain largely obscures the details of structure. All the three forms have been found in each specimen of the five infected fish.

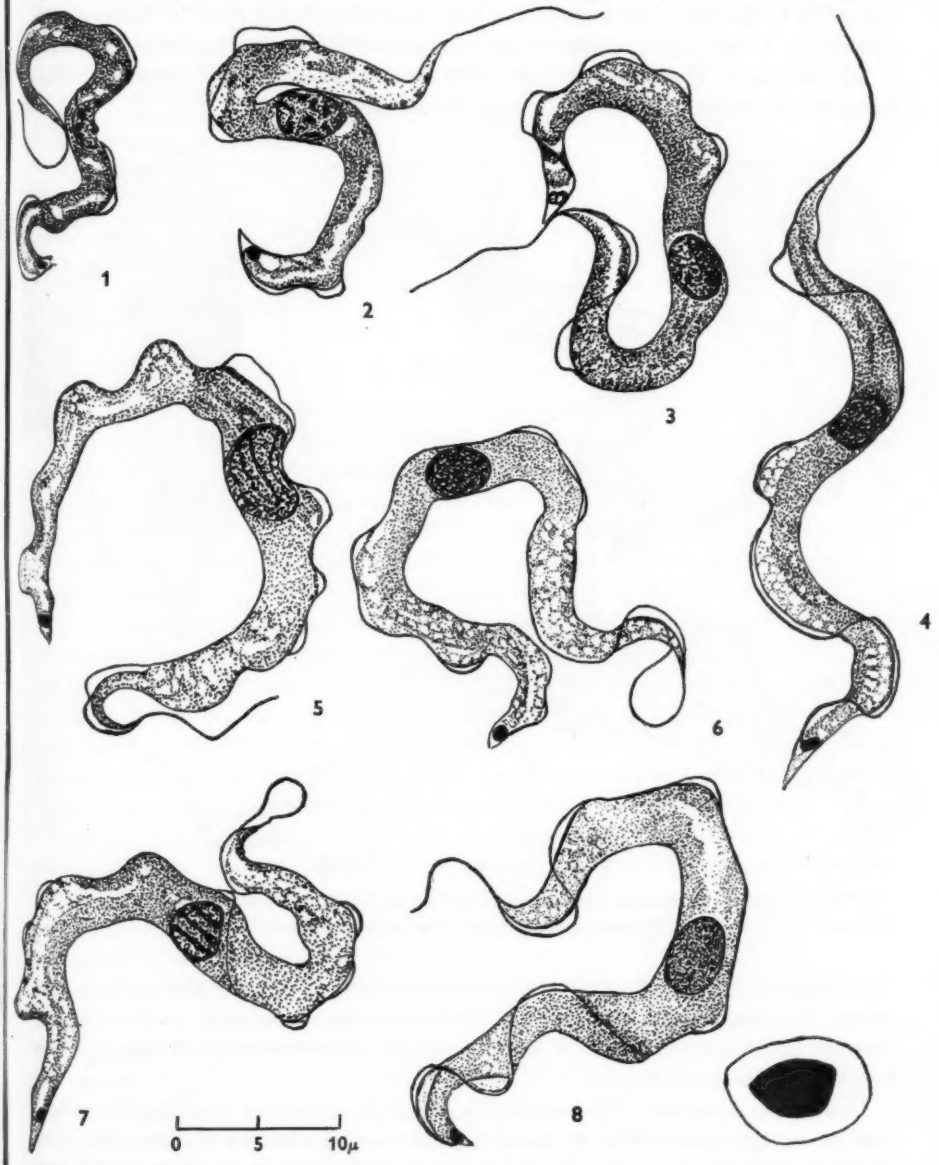
Stages of division were not observed in living preparations nor in the stained films.

Shape. The body of the flagellate as found in the host has a sinuous, elongated and fusiform shape. The anterior end is attenuated and pointed; the posterior end is less so and terminates rather abruptly in a more or less rounded extremity.

Cytoplasm. The cytoplasm in the smaller form, as mentioned above, is usually densely granular with very few vacuoles, but in the larger forms it is less granular and contains many vacuoles. The granules are rather irregular in shape and tend to cluster together in a compact mass. In the type which is intermediate in size these characters are also intermediate in degree.

Nucleus. The nucleus generally lies in the anterior half of the body. It is dark red in small forms and often very difficult to differentiate from the surrounding densely stained cytoplasm (Fig. 1). In large stout forms it is very clearly defined

with light staining. Generally the nucleus is spherical or oval (Figs. 8–10), or pear-shaped (Fig. 11), or bean-shaped (Figs. 5, 12). Its chromatin granules vary in size according to the size of the organism, being much larger in stout forms. These granules are either scattered or arranged in rows (Figs. 5, 7). This arrangement is

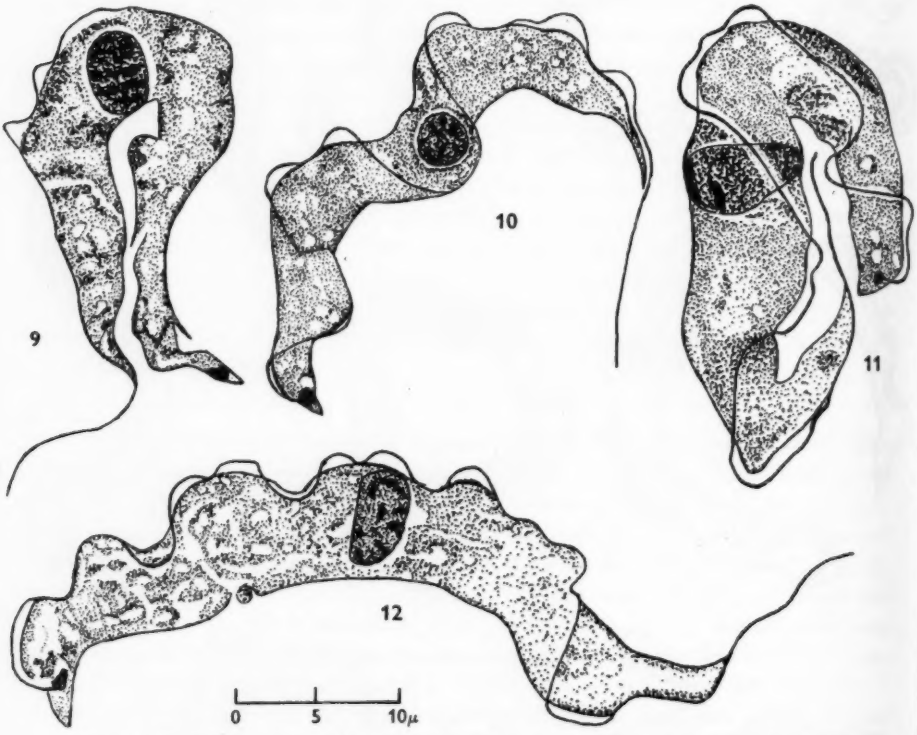


Figs. 1–8. *Trypanosoma striati* n.sp., from the blood of one specimen of the fish, *Ophicephalus striatus*. Preparations dried, fixed with methyl alcohol and stained by Giemsa's method. 1, small form; 2–8, intermediate forms.

not clearly seen in the small forms, as it is difficult to distinguish the granules owing to the deep staining of these forms. In some specimens a distinct karyosome, stained deeper red than the rest of the nucleus, is present.

Kinetoplast. The kinetoplast appears to be oval or rounded. It varies in size according to the size of the parasite. It lies a short distance in front of the posterior extremity. In rare cases a distinct large vacuole is seen surrounding the kinetoplast.

Blepharoplast. The blepharoplast was not observed in any specimen. The axoneme appears to arise directly from the kinetoplast.



Figs. 9–12. *Trypanosoma striati* n.sp., large forms, taken from the same blood and prepared in the same way as in Figs. 1–8.

Flagellum. The flagellum seems to originate from the kinetoplast. It is seen clearly running along the border of the undulating membrane and there is a distinct free flagellum, which is longer in small and intermediate forms, than in large and stumpy ones.

Undulating membrane. The undulating membrane is narrow in all the forms and has five to eight small folds. It does not become wider with the increase in the size of the body of the parasite. In a few cases the folds in the narrow undulating membrane are very long (Fig. 4), but usually they are short in all the forms. The membrane is completely hyaline in structure.

Dimensions. The average dimensions of each of the three forms of this trypanosome are given in Table 1.

Table 1

No.	Particulars	Small forms (μ)	Intermediate forms (μ)	Large forms (μ)
1	Posterior extremity of the body to posterior border of the nucleus	13·0	15·5	26·0
2	Posterior to anterior border of nucleus (length of the nucleus)	2·5	3·75	5·75
3	Anterior border of nucleus to anterior extremity of the body	16·5	19·75	23·0
4	Length of free flagellum	8·0	10·25	11·0
5	Total length of the body including flagellum	40·0	49·25	65·75
6	Breadth of the body at its widest part	2·0	3·0	8·0
7	Breadth of the nucleus	1·5	2·5	7·0
8	Length of the kinetoplast	0·75	1·0	1·75

IV. DISCUSSION

Lingard (1904) reported the presence of trypanosomes in *O. striatus* and in other species of fishes obtained from a river at Poona, South-west India. Morphological characters, dimensions and diagrams of the parasites were not given by him. He merely mentions 'In every instance the most marked development of the undulating membrane was observed.' In the same species of *Ophicephalus* from the River Jumna and its backwaters, Northern India, he reported another species of trypanosome, about which he only mentions that it was large in size.

The paucity of morphological data in Lingard's description of the unnamed species makes it very difficult to compare the trypanosomes observed by him with the present one. One point—the marked development of the undulating membrane —which he emphasized is, however, in itself sufficient to differentiate his species from the one described here, as the undulating membrane of the latter is not so strongly developed. Incidentally, what Lingard considered as two species of *Trypanosoma*, are most probably the dimorphic forms of one species only.

Wenyon (1908) recorded the presence of a trypanosome in the blood of *Ophicephalus obscurus*, from the River Nile. He stated that this trypanosome measured 40μ in length, 3–4μ in breadth, while the flagellum was about 4μ long. The kinetoplast (his micronucleus) was situated at the extreme posterior end and the nucleus was behind the middle point of the body.

The present trypanosome can be differentiated easily from this unnamed trypanosome of *O. obscurus* on the basis of the marked differences in the dimensions of the parasites, the situation of the kinetoplast, the topographical disposition of the nucleus in relation to the length of the body of the parasite and in the size of the free flagellum.

Mathis & Léger (1911) reported the occurrence of trypanosomes in *O. striatus* and *O. maculatus* from Tonkin. They gave a brief account of the parasites with dimensions but without nomenclature and without figures. The trypanosome found by them in *O. striatus* is described as being of small size with a long flagellum,

the protoplasmic body vacuolated, and the folds of the undulating membrane numbering 5 or 6.

Of the three forms of the trypanosome described by me, the small form has a shorter flagellum, the protoplasmic body sparingly vacuolated and the folds of the undulating membrane numbering 5 to 7. The dimensions of the internal parts and the body given by Mathis & Léger differ considerably from those of my recordings. A close scrutiny of the data of the two descriptions reveals that the species described by me is different; moreover, they have not recorded any polymorphism in the species described by them. As regards the trypanosome of *O. maculatus* it can be noted that the dimensions of this parasite and the absence of any indication of polymorphism differentiate the parasite observed by them from the one described by me. The latter was also compared with other species of trypanosomes recorded from various species of fishes in the literature and was found to differ, wherever an adequate account made such a comparison possible.

Taking all the foregoing facts into consideration it is proposed to give to the trypanosome from the fish, *Ophicephalus striatus* Bl., found in Hyderabad, India, the name *Trypanosoma striati* n.sp., with the specific characters as described in this account.

The work was carried out under the supervision of Professor H. E. Shortt, C.I.E., M.D., D.Sc., D.T.M. & H., F.R.S., I.M.S. (retd.), Director of the Department of Parasitology, London School of Hygiene and Tropical Medicine, University of London, to whom I wish to express my sincere indebtedness for his keen interest, continuous guidance, help and encouragement during the course of my work. I am grateful to Dr C. A. Hoare, F.R.S., for kindly editing this paper for publication. I owe my thanks to Mr W. Cooper, chief technician, Department of Parasitology and Mr R. Killick, technician, and to Miss G. Woosnam, assistant technician, for their help in the practical work. I am also thankful to Mr Bush, school photographer, for taking photographs of my drawings.

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THE INFECTION OF THE VENTRICULUS OF THE ADULT HONEYBEE BY *NOSEMA APIS* (ZANDER)

By L. BAILEY

Rothamsted Experimental Station

(With Plate IV and 3 Figures in the Text)

INTRODUCTION

The infection of the ventriculus of the honeybee by *Nosema apis* was studied as part of a programme of work devoted to the eventual development of control measures for the disease. It has already been shown (Bailey, 1952) that the spores of the parasite, after ingestion by the bee, are rapidly filtered out of the crop by the proventriculus and are then passed into the ventriculus.

Further work has been carried out to try to establish: (1) the cause of germination of the spores within the ventriculus; (2) the method by which the parasite enters the ventricular epithelium; and (3) the phenomena associated with its development within the epithelium. These investigations are discussed in the three sections below.

METHODS

Stock preparations of spores were obtained by grinding up ventriculi and rectums of infected bees in water, filtering through bolting silk, centrifuging down the spores from the filtrate and resuspending them in approximately 66% sucrose solution. These spore suspensions seem to maintain viability indefinitely when kept in a refrigerator between 0 and 5° C.

Bees were individually fed with definite numbers of spores by enclosing the bee in a specimen tube fitted with a cork which held a small glass cup. The requisite volume of spore suspension (usually about 0·02 ml.) was injected into the cup with an 'Agla' microsyringe. Suitably starved bees usually ate the whole volume within an hour.

I. THE CAUSE OF GERMINATION

The digestive fluid of the intestine has usually been described as the stimulus causing germination of the spores of the Microsporidia, although Morgenthaler (1922) said that the spores of *N. apis*, if ripe, were capable of discharging their polar filament even when suspended in distilled water. Gibbs (1953) successfully caused the filaments to extrude from some of the spores of a *Gurleya* species by dilution with saline after a period of desiccation. Ohshima (1937) noted the extrusion of filaments from spores of *Nosema bombycis* when they were mixed with the digestive fluid of silkworms even if the fluid had been boiled. Thus he excluded digestive enzymes as the stimulus. Trager (1937) was unable to repeat Ohshima's results but saw filament extrusion when he mixed spores with a liver extract medium if the pH was 8·0 or more. Ohshima (1937) also saw filament extrusion if

a spore suspension at pH 13·0 was reduced to neutrality with HCl. Other artificial agents causing filament extrusion include hydrogen peroxide, dilute acids, iodine solutions and mechanical pressure. Such a diverse assembly of stimuli have little in common and the possible agencies operating *in vivo* remain somewhat obscure. Experiments were conducted therefore to try to determine the probable natural agencies causing filament extrusion in *Nosema apis*.

Experiments

First the relationship between the number of viable spores which had been fed to individual bees and the number of spores which subsequently developed after 7 days was established (Text-fig. 1) by counting the spores which had developed in individual bees. This was done by grinding up the ventriculi of individual bees in a convenient volume of water (usually 1 ml.) and assessing the spore concentration by means of a haemocytometer.

Spores from the stock suspensions were then subjected to experimental conditions, which were considered to be likely causes of germination *in vivo*, and were then quantitatively fed to individual healthy bees. Control bees were similarly fed with untreated spores, and the degree of infection of individuals from the two groups of bees was established after 7 days. Thus any diminution of viability of the treated spores would be expressed by a reduction of the number of spores developed in the individual bees. Since the same level of infection was produced by feeding any number in excess of one million viable spores per bee (Text-fig. 1) less than one million spores were fed per bee in these experiments. Any slight but significant diminution of viability might thereby still be detected.

Spores were then treated in the following ways:

(*a*) Spores were fed to healthy bees and, after periods of 30, 60 and 120 min., the ventriculi of some of the bees were dissected out, ground up in sucrose syrup, checked for concentration of spores and then quantitatively re-fed to further healthy bees, each bee receiving about 16,000 to 20,000 spores.

(*b*) A spore suspension in syrup was diluted rapidly with distilled water. Thus the osmotic pressure of the medium was reduced from about 100 atmospheres (which is roughly equivalent to that of the honey in the honey stomach of the bee) to nearly nil (which is approximately the pressure of the ventricular contents).

(*c*) The oxygen tension of a watery suspension of spores was reduced by enclosing it in a sealed container with alkaline pyrogallol.

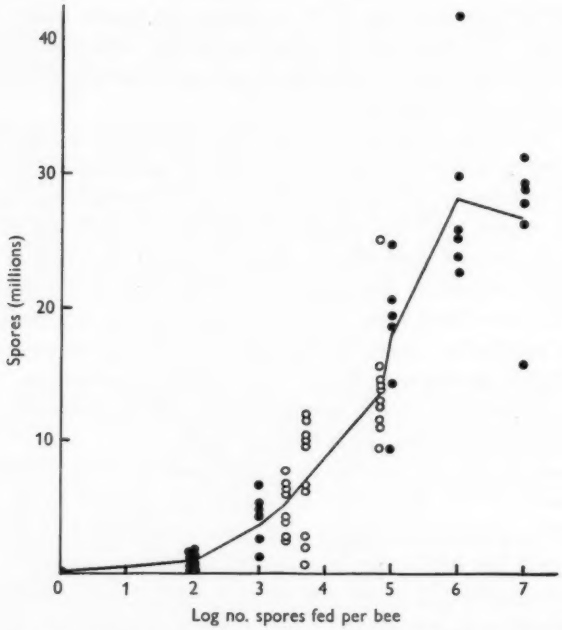
(*d*) The pH of spore suspensions was changed rapidly from pH 4·0 (approximately the pH of honey) to pH 6·0 and more (approximately the pH of the ventricular contents) by dilution with phosphate buffers.

(*e*) Spores were mixed with extracts of ventriculi of healthy bees buffered at various levels from pH 6·0 to 8·0 for 24 hr. or more. The ventricular extracts were kept as concentrated as possible by adding only the minimum quantities of buffers.

(*f*) Spores were injected directly into ventriculi freshly extracted from bees by means of a fine hypodermic needle. The needle could be inserted easily into the anterior end of the organ and slid into a central position within the peritrophic membranes. Its position could be seen through the translucent tissue when it was

brilliantly illuminated. The spores were injected in the minimum quantity of fluid (either 0·5 or 1·0 mm.^{3}). After incubation at 30° C. for 8 hr. the ventriculi were ground up in syrup and quantitatively fed to healthy bees.

This experiment was repeated with spores which had been injected into ventriculi after they had spent a few hours in the crops of bees. These bees had been fed with concentrated spore suspensions and their crops were then dissected out, after about 10 min., followed by incubation for a few hours at 30° C. In this instance, therefore, the spores which were finally quantitatively fed to the individual healthy bees had been pre-treated by the digestive fluids of the crop as well as those of the ventriculus.



Text-fig. 1. The relationship between the number of spores of *N. apis* fed to individual honeybees and the number of spores which develop per bee after 7 days. The open circles show the results obtained by using a different stock of spores (prepared on a separate occasion) and illustrate their fairly consistent quantitative nature.

Results

Only those spores which had been treated by the method described in (*a*) above had suffered a reduction in viability.

The mean level of infection developed by the bees which were fed untreated (control) spores was 10·05 million per bee (average of eight bees). The mean levels of infection developed by bees which had been fed with spores which had been in the ventriculi of living bees for 30, 60 and 120 min. were 1·70, 2·85, and 0·25 millions per bee respectively. All the treated spores had had their viability reduced significantly ($P < 0·01$) below that of the control spores. The spores which had been

in the ventriculi for 120 min. had had their viability reduced significantly ($P < 0{\cdot}001$) below that of all the other groups.

These different levels of infection were compared with each other by means of Fig. 1. The level of infection produced by the spores which had previously been in ventriculi for 30 or 60 min. was approximately equal to the infection produced by about 250–800 viable spores. In other words, the viability of the 16,000–20,000 spores which had been fed to these bees had been reduced by 95–99 %.

Discussion

It seemed likely that the great majority of spores germinate almost immediately after their entry into the ventriculus since it takes about 30 min. for all the spores to be filtered out of the crop into the ventriculus (Bailey, 1952).

The failure of the last method (*f*), described above, to cause germination was particularly interesting since the conditions were as near *in vivo* as possible without using live bees. It suggests that the cause of germination is either complex or is a stimulus which is easily deranged under experimental conditions.

II. THE ENTRY OF THE PARASITE INTO THE VENTRICULAR EPITHELIUM

A widely accepted account of the method by which the Microsporidian parasite enters the ventricular epithelium of the insect host has been given by Stempell (1909), Fantham & Porter (1912), Kudo (1924), Doflein & Reichenow (1953) and many others. It is said that the polar filament of the spore is extruded under the influence of the digestive fluid of the host and serves as an anchor for the spore by attaching itself to the ventricular epithelium. The filament is then said to break away and a minute amoeboid 'planont' is said to emerge into the ventricular lumen from the pore left in the spore capsule and to make its way by slow amoeboid movements on to the ventricular epithelium where it penetrates the cells and develops intracellularly. Since the alleged planont is said to be minute (1–2 μ diameter) and slow moving it might be expected that the initial infection of the ventriculus would be at or near the posterior end since matter which is passed into the organ from the crop is deposited well into the lumen at the end of the oesophageal invagination (Pl. IV, fig. 1) and within the concentric layers of peritrophic membranes. Food then reaches the posterior end of the ventriculus after about 1 hr. (Bailey, 1952). However, it was noticed in a longitudinal section of a recently infected ventriculus that the anterior end of the organ seemed to be the most heavily infected part.

Experiments were conducted to examine this apparent anomaly further.

Experiments

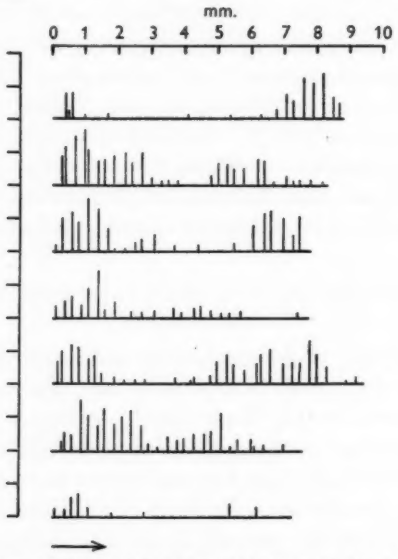
Seven individual bees were infected in the manner described above. Each bee received over a million spores thereby receiving a saturation dosage of infective material (Text-fig. 1).

After 48 hr. the ventriculi were extracted and were fixed in Bouin's fixative. The organs were fixed by allowing them to adhere longitudinally to the wall of

a small tube containing fixative and then tilting the tube so that the fluid just contacted the organ. After a few seconds the ventriculi were allowed to settle into the fluid and they then remained quite straight.

Longitudinal sections were cut as near as possible to the mid-line of each ventriculus, and the preparations were stained with Heidenhain's iron haematoxylin.

Counts of the number of infected cells per fluid of view under a $\frac{1}{6}$ in. objective were then made starting at the anterior end of each section and working down one side to the posterior end.



Text-fig. 2. The distribution of the initial (48 hr.) infection of the ventriculus of the honeybee by *N. apis*. Each horizontal base line represents the length of one ventriculus with the anterior end to the left. The vertical lines rising from the base-lines represent the number of infected cells per field of view under a $\frac{1}{6}$ in. objective. The divisions on the vertical scale are each equivalent to ten cells. The horizontal arrow represents the average length of the oesophageal invagination and its membranes (cf. Pl. IV, fig. 1).

Results

These are portrayed in Text-fig. 2. It is evident that there is usually a high degree of initial infection at the anterior end of the ventriculus. Sometimes this anterior infection is actually greater than that at the posterior end. There is also a central region of the ventriculus where the initial infection is relatively low.

Discussion

It is considered that these results give support to the suggestions of some workers that the infective germ of the Microsporidian spore does not normally exist as a free 'planont' but is injected directly into the host cell from the spore via the hollow polar filament. Korke (1916), Ohshima (1937) and Gibbs (1953) who observed a nucleated drop of protoplasm issuing from the distal ends of the artificially extruded filaments of *Nosema pulicis*, *N. bombycis* and a *Gurleya* species respectively,

each suggested that this nucleated mass was the parasite which normally would be injected into the host tissue pierced by the polar filament. A fluid mass, sometimes described as nucleated, has also been described as issuing from the artificially extruded filaments of *N. apis*, *Stempellia magna* and *N. bombycis* by Morgenthaler (1922), Kudo (1924) and Trager (1937), although no conclusion was drawn by these authors about the phenomenon. Kudo (1924) cast some doubts upon the 'planont' theory when he says 'the entire process of emergence of a living amoebula from a spore has unfortunately not been observed in any case'. He also remarks (Hegner & Andrews, 1930) that the amoeboid movement of the 'planont' seems to have been deduced from the irregular shapes of fixed and stained specimens.

The high degree of anterior infection of the ventriculus of the bee described above is understandable by the theory of injection of the parasite by the filament which is undoubtedly capable of piercing the multi-layers of peritrophic membranes as soon as the spore enters the ventriculus (Pl. IV, fig. 1). The route for a minute 'planont' would be exceedingly tortuous demanding relatively great speed and agility, otherwise it would be swept along to the posterior end of the ventriculus with the food. Furthermore, the cells containing developing spores are generally discrete with healthy tissue on either side, particularly in the initial stages of infection (Pl. IV, fig. 2), and there is no hint of a spread of infection from primarily infected centres where 'planonts' might have found their way through to the epithelium.

Morgenthaler (1922) gives the length of the polar filament of *N. apis* as about 400μ and it is likely that this is a minimum figure. It is quite feasible, therefore, for the filament to reach the tissues from within the peritrophic membranes. 'Planonts', which have been described in the lumen of the ventriculi, have probably been parasites which have arisen from filaments which have failed to reach or penetrate the epithelium.

III. THE INTRACELLULAR DEVELOPMENT OF THE PARASITE

A phenomenon accompanying the intracellular development of the parasite is the corresponding reduction in number of the spherical granules of $1–3\mu$ diameter which are found in very large numbers in the outer edge of the ventricular epithelium (Pl. IV, fig. 3).

Köhler (1920) concluded that the granules were composed of calcium carbonate, although there was no positive evidence for the carbonate radical. Day (1949) noted that the granules contained phosphate, and Waterhouse (1951) noted that they contained barium and strontium. The distribution of these granules in healthy bees was examined, and the inorganic phosphate content of the ventricular lumen in healthy and diseased bees was estimated to try to establish the significance of the granules to the developing disease. The solubility of the granules at different levels of pH was also examined.

Experiments

The distribution of the granules in healthy ventriculi was examined by fixing the organs as straight as possible by the method described in § II, in 80% alcohol. Longitudinal sections were made as near to the mid-line of the ventriculi as possible

and the preparations were tested for inorganic phosphate by washing successively with calcium nitrate, cobalt nitrate and ammonium sulphide as in Gomori's technique (Danielli, 1945).

Estimates of inorganic phosphate in the lumen of healthy and diseased ventriculi were made by extracting samples of the contents of the organs in the following way. Ventriculi were extracted from healthy or diseased bees, washed in three changes of distilled water, dried by rolling gently on filter-paper and then split open at one end. A thin glass rod was rolled gently along the length of the organ and the contents were thereby expelled on to a waxed slide. The viscous fluid was then taken up into a graduated capillary tube by gently tapping the slide through the drop of fluid with the end of the tube. Sixteen samples, each of 8·1 mm.^{3} of ventricular fluid were taken from two groups of bees. Eight samples were from a group heavily infected with *Nosema* and eight were from a healthy group. The ventricular fluid of at least two bees contributed to each sample.

Each sample was washed into 2·5 ml. of distilled water to which 2·5 ml. of 2N-H_2SO_4 was added. The mixture was then filtered through Whatman no. 5 paper. To 4 ml. of filtrate was added 0·5 ml. of 7·5% ammonium molybdate solution and 0·5 ml. of a freshly prepared 0·2% solution of stannous chloride. The blue colour which developed was estimated on an 'Eel' photo-electric colorimeter.

The solubility of the granules at different levels of pH was estimated as follows. 0·2 ml. of a suspension of healthy ventriculi (prepared by grinding up 10 ventriculi in 1 ml. of distilled water) were mixed with volumes of 0·8 ml. of a series of 0·1M phosphate buffers. The mixtures were boiled, allowed to cool, and examined microscopically after a few hours.

Results

The distribution of the granules in the ventriculus is shown diagrammatically in Text-fig. 3. The granules stained black by Gomori's technique even if the calcium nitrate wash was omitted. It seemed likely then that they were largely composed of calcium phosphate. They probably contain little or no organic matrix since they were seen to dissolve and disappear without trace when acid was allowed to run into a smear preparation of the ventriculus under microscopic observation. No staining was observed in ventriculi which were infected with *N. apis*.

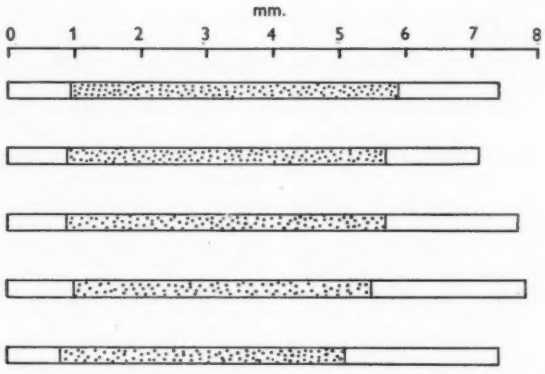
The mean concentration of inorganic phosphate in the contents of healthy ventriculi was 0·0073M. The mean concentration in diseased ventriculi was 0·0165M. These means were significantly different ($P < 0·01$).

The granules were found to dissolve completely *in vitro* at a pH of 5·9 or less. They were apparently unchanged when the pH was at 6·3 or more. It was fairly definite that they were fewer and smaller at pH 6·0 than at pH 6·2.

Discussion

The dissolution of the phosphate granules as the infection develops and the raising of the concentration of inorganic phosphate within the lumen of the diseased ventriculus suggests that the material of the granules is passing in solution into the ventricular lumen.

The initial low degree of infection in the central region of the ventriculus (Text-fig. 2) may be correlated with the distribution of the granules. The mean number of infected cells in the anterior, granule free, millimetre of the ventriculi represented in Text-fig. 2 was 29·3, whereas the mean number of infected cells per millimetre in the following granular region, whose average length is 4·8 mm., was 12·2. These means were significantly different ($P < 0{\cdot}01$). It may be therefore that the development of infection is initially inhibited by the granules. The mechanism of this possible inhibition is suggested by the solubility of the granules at different levels of pH.



Text-fig. 3. The distribution of the granules of calcium phosphate down the length of a healthy ventriculus of the honeybee. Each block represents one ventriculus with the anterior end on the left. The stippled sections represent the granular region.

The pH of the contents of the normal ventricular cell may be slightly too high for the developing parasite, and it may be adjusted quite quickly to a lower value as the parasite begins to develop. However, the pH of the cytoplasm of the granular cells will be strongly buffered against a lowering of pH below 6·3 and so may inhibit the rapid development of the parasite until the granules have been dissolved away.

GENERAL SUMMARY

1. Spores of *Nosema apis* nearly all germinate within 30 min. after entering the ventriculus of the honeybee.

2. The cause of germination *in vivo* remains unknown, but is probably not a change of osmotic pressure or pH and is probably not due to anaerobic conditions or an enzyme. The agent causing germination is probably unstable and is destroyed when removed from the bee.

3. The anterior end of the ventriculus receives an initial heavy infection and the circumstantial evidence which this provides supports the theory that a free living 'planont' stage does not exist but that the parasite is injected directly into the host cell from the spore via the hollow polar filament.

4. The central region of the ventriculus which contains many granules of calcium phosphate receives the lowest initial infection. It is considered possible

that the granules inhibit the initial rapid development of the parasite by maintaining an unfavourably high level of pH until they are eventually dissolved by the slowly developing organism.

I wish to thank Dr C. G. Butler for his help with the photography, Mr J. Adams for technical assistance, and Miss M. Holden of the Biochemistry Department for kindly giving facilities and advice for inorganic phosphate analysis.

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EXPLANATION OF PLATE IV

Fig. 1. Longitudinal section of the anterior part of the ventriculus of the honeybee. Bouin and Heidenhain's iron haematoxylin. *O*, oesophageal invagination; *P*, multi-layers of peritrophic membranes; *E*, ventricular epithelium; *X*, point at which material, passing down the oesophageal invagination from the crop, enters the ventricular lumen.

Fig. 2. Ventricular epithelium of honeybee with initial infection of *N. apis*. Bouin and Heidenhain's iron haematoxylin. *C*, infected cell containing developing spores; *H*, healthy cells on either side; *P*, layers of peritrophic membranes in ventriculus.

Fig. 3. The intracellular calcium phosphate granules of the ventricular epithelium. 80% alcohol and Gomori's treatment for inorganic phosphate. *G*, granules of calcium phosphate (stained black); *L*, ventricular lumen; *M*, circular muscles surrounding ventriculus.

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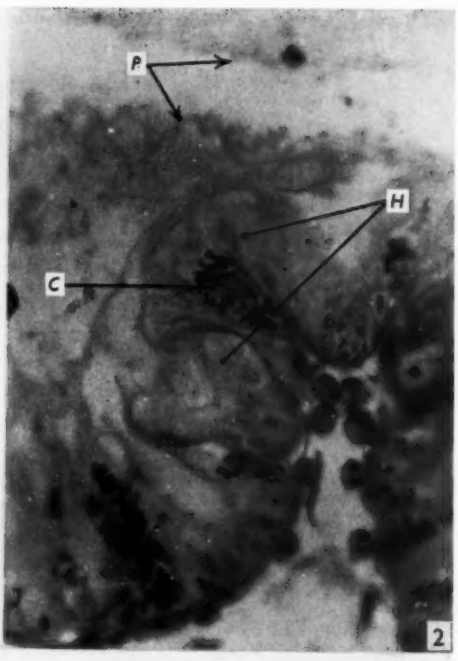

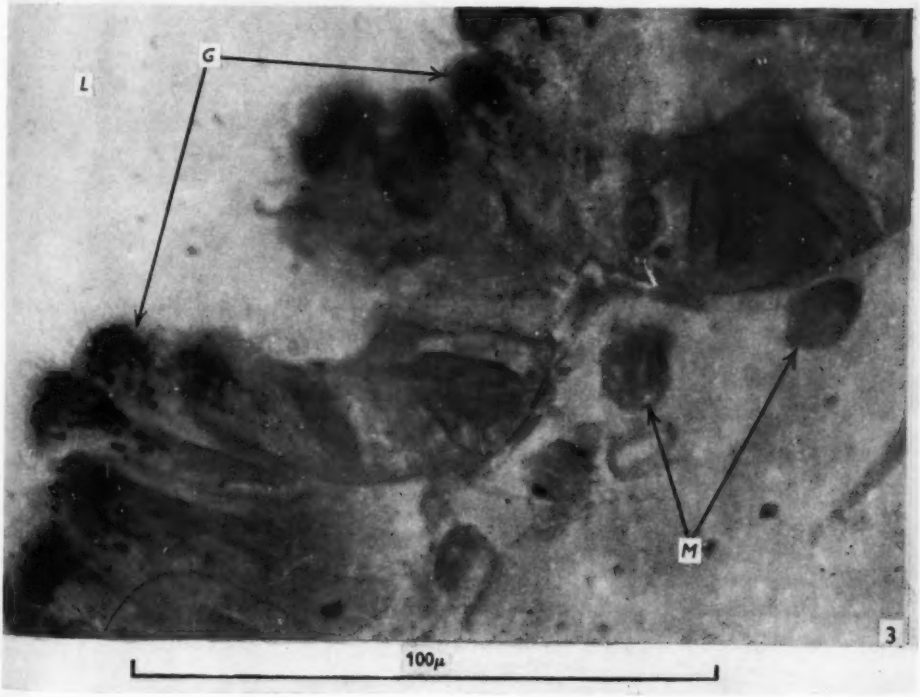

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RHIPICEPHALUS PULCHELLUS GERSTÄCKER 1873: A DESCRIPTION OF THE LARVA AND NYMPH WITH NOTES ON THE ADULTS AND ON ITS BIOLOGY

By JANE B. WALKER

East African Veterinary Research Organization,
P.O. Box 32, Kikuyu, Kenya Colony

(With 7 Figures in the Text)

INTRODUCTION

Rhipicephalus pulchellus is an easily recognizable species of tick which appears to be confined to Eastern Africa. The larvae and nymphae, which are described here for the first time, and the adults, on which notes on morphological variation are given, are the progeny of a single female. This female was collected from a bovine at Kiboko (101 miles from Nairobi on the road to Mombasa) by Dr S. G. Wilson and her progeny were reared in the laboratory.

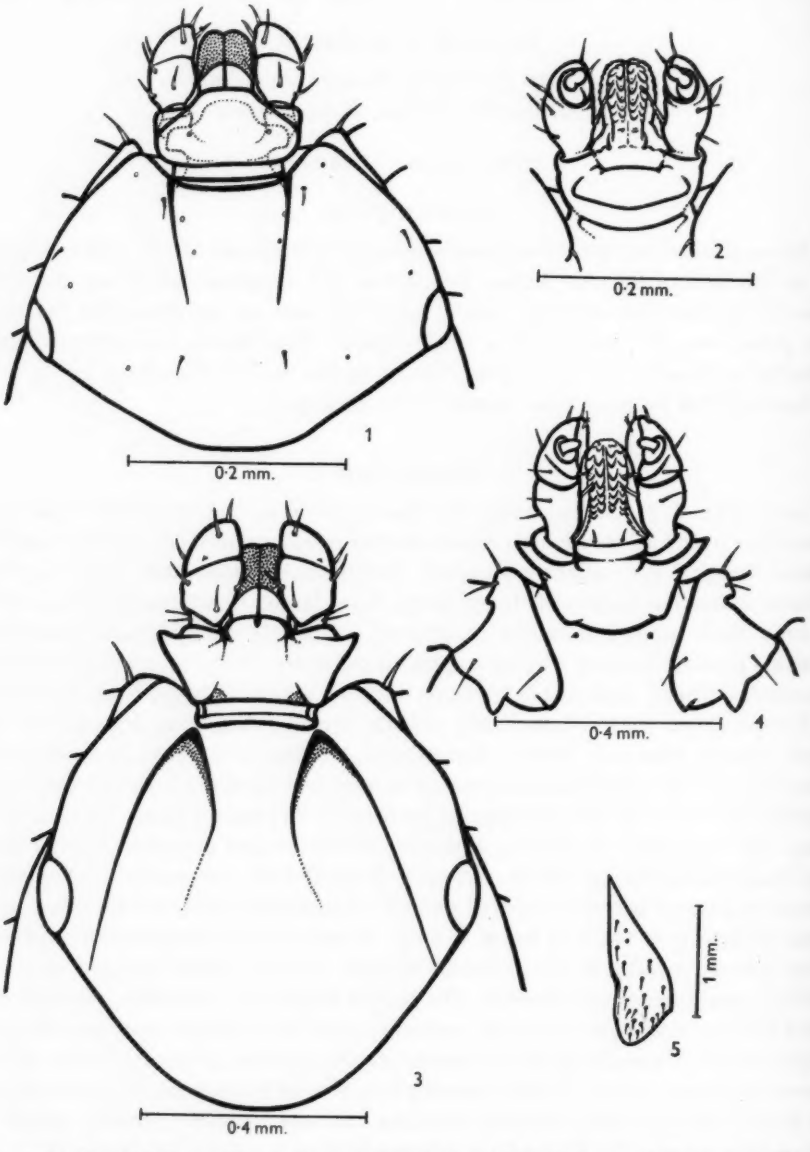
DESCRIPTION

Larva (Figs. 1–2). *Scutum* about 1½ times as broad as long, broadest at eye level, somewhat over half-way back. Antero-lateral margins smoothly curved; postero-lateral margins very slightly sinuous. Emargination wide and deep. Cervical grooves extending backwards to eye level. Eyes large. *Capitulum* about as broad as long. *Basis capituli* almost twice as broad as long, right-angled antero-laterally, rounded postero-laterally. No spurs present ventrally. *Palps* broad with smoothly rounded contours. Article I just visible dorsally, article II larger than article III.

Nymph (Figs. 3–4). *Scutum* only slightly broader than long, broadest at eye level, nearly half-way back. Antero-lateral margins convex; postero-lateral smoothly curved. Emargination rather narrow and shallow. Cervical pits deep; cervical fields sunken and demarcated by cervical and lateral folds. Cervical folds deep and convergent anteriorly, becoming shallower and divergent further back and finally disappearing opposite the eyes. Lateral folds well marked and reaching almost to postero-lateral margins of scutum. *Capitulum* slightly broader than long. *Basis capituli* over twice as broad as long. Antero-lateral margins short and concave, almost parallel to the posterior margin; postero-lateral margins concave, much longer than antero-laterals. The lateral angles are, therefore, very far forward and are sharp and recurved ventrally. Posterior margin straight with very slight cornua. Ventrally spurs are present at the junction of the auriculae and the curved posterior margin. *Palps* normally overlap the hypostome, not quite as long as broad with smoothly rounded contours. Article I visible dorsally, article II larger than article III. Ventrally a retrograde spur is present on article III.

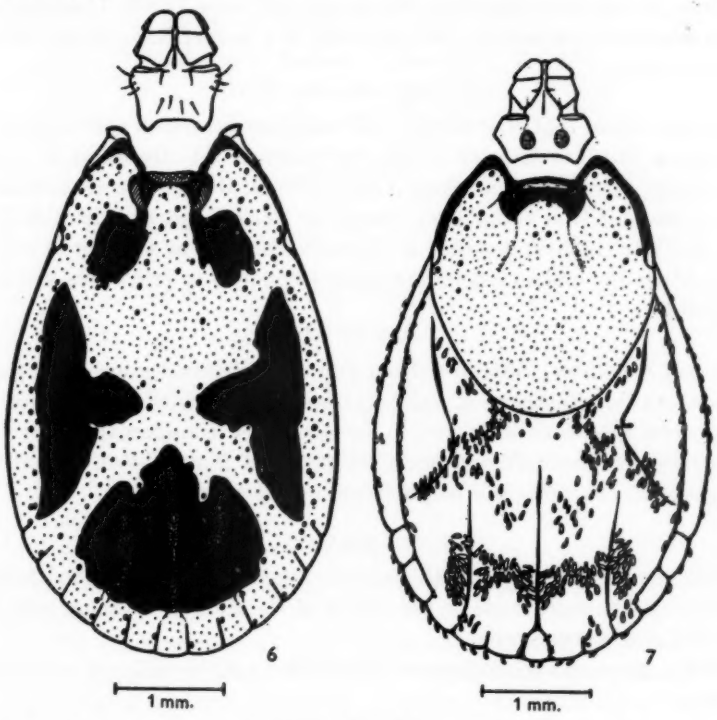
Adults (Figs. 5–7). The adults of this species have been redescribed twice in recent years, by Zumpt (1941) and Theiler and Robinson (1953), and the variations

in their size and ornamentation as seen in field collections have been carefully analysed by Cunliffe (1913). A detailed description of the adults in this series, of which Figs. 5–7 represent typical examples, will not, therefore, be made. The males vary in size from 4·6 × 3·2 to 2·5 × 1·5 mm., but show a striking uniformity of



Figs. 1–5. *R. pulchellus*. Fig. 1. Larva—dorsal view of capitulum and scutum. Fig. 2. Larva—ventral view of capitulum. Fig. 3. Nymph—dorsal view of capitulum and scutum. Fig. 4. Nymph—ventral view of capitulum. Fig. 5. Male—anal plate.

pattern (Fig. 6). Only in one or two specimens is there a tendency for slight fusion of the lateral dark patches with the postero-median dark patch, and in no case does the ornamentation approach either of the extreme forms illustrated by Cunliffe. The females are much more uniform in size than the males and only vary from 4·2 × 3 to 3·7 × 2·5 mm. Their ornamentation and punctation patterns are also very constant (Fig. 7).



Figs. 6–7. *R. pulchellus*. Fig. 6. Male—dorsal view of capitulum and conscutum. Fig. 7. Female—dorsal view of capitulum, scutum and alloscutum.

DEVELOPMENTAL PERIODS

Apart from feeding, which was on rabbits kept at air temperature, all stages of development took place in an incubator at 25–27° C. with high humidity. Accurate records of the period required by each stage for hardening (this period is frequently taken to be approximately 1 week) were not kept, but the average periods required for other phases of development were as follows:

	Days		Days
Pre-oviposition	6	Nymphae harden	—
Larvae hatch	39	Nymphae engorge	3
Larvae harden	—	Nymphae moult	15
Larvae engorge	3	Adults harden	—
Larvae moult	11	Adults engorge	11

No difficulty has been experienced in breeding this tick as all stages feed readily on the ears of rabbits. Montgomery (1913) and Lewis (1932) reported they had been unable to feed larvae on cattle. The author has found that all stages of the tick will feed on the ears of cattle but not so readily as they do on rabbits.

HOSTS

In addition to the hosts listed by Theiler and Robinson (1953) Thomson's gazelle (*Gazella thomsoni* Günther) has been recorded as a host of *R. pulchellus*, principally of the nymphae.

DISEASE TRANSMISSION

Lewis (1949) found that *R. pulchellus* will sometimes transmit the virus of Nairobi sheep disease but it is not such an efficient vector of this disease as *R. appendiculatus*. Attempts by various workers (Lewis, Wilson, Barnett, personal communications) to transmit East Coast fever, caused by *Theileria parva*, with this tick have failed. Roberts (1935) states that *R. pulchellus* may act as a vector of tick typhus in East Africa but transmission experiments designed to prove this yielded negative results.

CLASSIFICATION

Theiler and Robinson (1953) in a discussion on the classification of *R. pulchellus*, comments on the subspecies *R. pulchellus humeralis* Rondelli. The present author, having reared specimens of this tick in the laboratory, considers that they represent a true species, *R. humeralis*, and not a subspecies of *R. pulchellus*. Descriptions of all stages of *R. humeralis* will be published in due course.

SUMMARY

1. The larva and nymph of *Rhipicephalus pulchellus* are described for the first time and comments are made on the degree of variation in structure seen amongst adults of a single generation.

2. Information on various aspects of the biology of this tick and on classification is given.

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NEMATODE PARASITE POPULATIONS IN SHEEP ON LOWLAND FARMS

II. WORM EGG COUNTS IN LAMBS

By H. D. CROFTON, B.Sc., Ph.D.
Department of Zoology, University of Bristol

(With 9 Figures in the Text)

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I. INTRODUCTION

This work started in May 1950 and is part of a wider study of parasite populations on lowland pastures.

An account of observations made on ewes has already been given (Crofton, 1954), and the present paper deals with observations made on lambs from the same flocks. It is realized that a proper approach to parasite problems in sheep entails consideration of the flock as a whole, but for convenience, the observations made on different age groups have been described separately. It is for convenience, also, that pasture contamination is only considered briefly here. Later an attempt will be made to consider parasite populations on a lowland farm as a dynamic system of interacting factors.

II. MATERIALS AND METHODS

From May 1950 to August 1953 observations were made on lambs in commercial flocks in the south-west of England. The flocks have already been described (Crofton, 1954). Lambs were marked shortly after birth by tattooing their ears. Each lamb could then be identified individually, and when samples of faeces were taken from the rectum the number of the lamb was recorded on the container. The techniques used for worm egg counts and differential larval counts were the same as those described previously (Crofton, 1954; Cunliffe & Crofton, 1953).

III. RESULTS

The figures in the diagrams are all calculated in the usual way and represent the mean number of eggs per gram (e.p.g.) per sheep except where it is stated otherwise. To avoid confusion the term 'lambs' will be used even after weaning, when it would be more correct to use the term hogg.

(a) *General trends in egg counts* 1950–3

(i) *Flock A*

Throughout the whole period of observation the lambs received only one anthelmintic treatment, this being given in October 1950.

In 1950 (Fig. 1*a*) observation started in May. At first the counts were low, increasing slowly until the end of June. In July there was a rapid increase from less than 120 e.p.g. to more than 720 e.p.g. by the third week. At the end of the month there was a slight fall but it is doubtful whether this is significant. The number of eggs increased rapidly during late August and reached a maximum of 2650 e.p.g. by the middle of September. There was a very rapid decrease in October *before* treatment with phenothiazine was given. At the end of October there were less than 100 e.p.g. per lamb and the number remained low until the sheep were sold. From the end of October the sheep were folded on turnips, rape and kale. In 1951 (Fig. 1*b*) the observations started in April. The number of eggs remained low until autumn. There were less than 200 e.p.g. until the middle of July; from the beginning of August there was an increase and by the middle of September there were about 500 e.p.g.; a very rapid increase occurred in October and by the end of this month, the mean count was nearly 1800 e.p.g. This rise was followed by a rapid decrease until the middle of November. The counts then fell more gradually until, in January, when the observations ceased, there were only 200 e.p.g.

In 1952 (Fig. 1*c*) a similar pattern was followed but the rise was more rapid. The maximum count, although lower than in previous years, occurred early in August. After this time, the reduction was rapid and at the end there were less than 150 e.p.g. There was a slight rise to about 330 e.p.g. in October and November, but by January there were less than 50 e.p.g.

In 1953 the observations did not start until June. The maximum count of 1100 e.p.g. was recorded in October and although this was again a low maximum, the general pattern was similar to other years.

(ii) *Flock B*

During 1950 the lambs in this flock received anthelmintic treatment once a month, the first dose being given in June. The anthelmintics used were phenothiazine and copper and nicotine sulphate mixture.

Samples were first taken at the end of May, the initial count being 370 e.p.g. By the middle of June there were more than 700 e.p.g. Anthelmintic treatment was then given and there was a rapid fall in egg counts. By the end of July there were less than 200 e.p.g. Despite repeated dosing the egg counts rose to 500 e.p.g. and remained at this level until the end of October. There was then a gradual reduction,

but although anthelmintic treatment was given in November and December the egg count was still above 200 e.p.g. at the end of January (Fig. 2*a*).

In 1951 anthelmintic treatment was given on three occasions, twice in August and once in September. Counts started in April and rose from less than 100 e.p.g. to nearly 1200 e.p.g. by the end of July. After the first two treatments there was no further significant increase but the counts did not fall until the end of September when there were approximately 500 e.p.g. Further anthelmintic treatment was

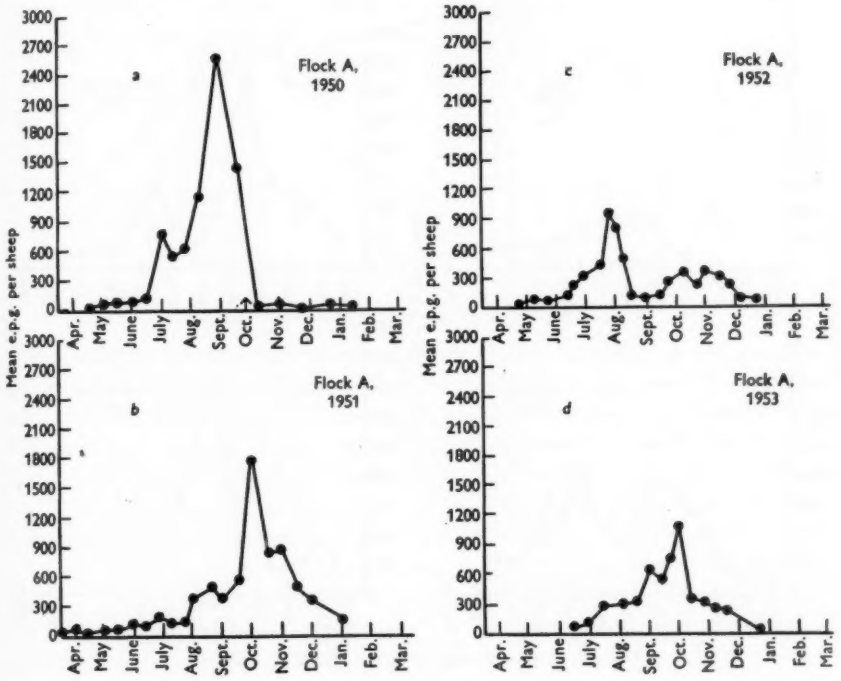

Fig. 1*a–d*. Worm egg counts. Flock A, 1950–3. E.p.g., eggs per gram of faeces per sheep.

given but this was followed by an increase in egg count. In the first week of November the highest count of the year (1200 e.p.g.) was recorded. There followed a gradual reduction but there were still more than 300 e.p.g. at the end of January when observations ceased (Fig. 2*b*).

In 1952 there were more than 300 e.p.g. on the first occasion of sampling at the end of April. Counts rose steadily and rapidly until the maximum count of nearly 2000 e.p.g. was recorded at the beginning of August. Thereafter counts fell rapidly there being less than 600 e.p.g. at the end of August. By the third week in September there were less than 300 e.p.g.—but the counts rose again, until at the beginning of November there were nearly 900 e.p.g. After this time the counts fell to about 150 e.p.g. for the rest of the year, remaining low until February when the observations ceased (Fig. 2*c*).

In 1953 there were again more than 300 e.p.g. at the end of April, but counts fluctuated between 250 and 650 e.p.g. until September when the highest count of the year was recorded. At this time there were more than 2100 e.p.g., the highest number recorded on this farm during the 4 years of observation. There was no opportunity to sample the flock again until the first week of October, and by this time the count had fallen to about 900 e.p.g. (Fig. 2*d*).

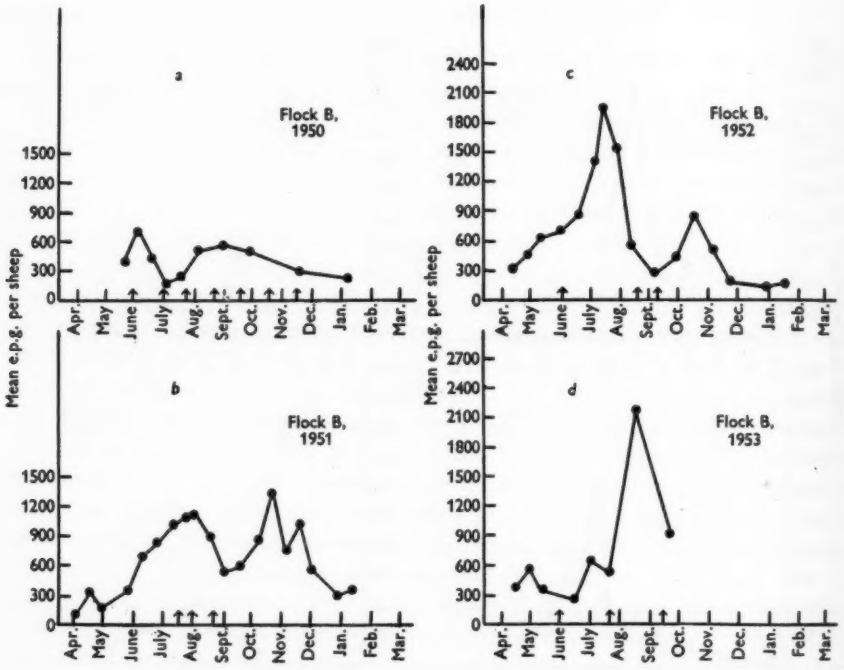

Fig. 2*a–d*. Worm egg counts. Flock B, 1950–3.

(iii) *Flock C*

Observations were made on the lambs in this flock for only two seasons, 1951 and 1952. Anthelmintic treatment was given regularly.

In 1951 the counts rose steadily from 100 e.p.g. in the middle of April to more than 1500 e.p.g. in August despite anthelmintic treatment commencing in June. Following two treatments in August the counts fell to 1200 e.p.g. but rose again to nearly 1800 e.p.g. at the end of September. The number of eggs fell gradually, but even at the beginning of December there were 900 e.p.g. After further anthelmintic treatment in December the counts fell to 300 e.p.g. by the third week in December. In the middle of January there were still nearly 300 e.p.g. On the last occasion of sampling, at the end of January, the count was more than 700 e.p.g. (Fig. 3*a*).

In 1952 observations were not commenced until mid-May, when the count was about 650 e.p.g. Counts rose steadily until, at the end of July, there were nearly

1500 e.p.g. Counts fell rapidly after two anthelmintic treatments in August. By the third week of September there were less than 150 e.p.g., but in the middle of October there were nearly 500 e.p.g. and by the first week in November there were more than 1200 e.p.g. Anthelmintic treatment given in November was followed by a decrease, there being less than 300 e.p.g. in the middle of January (Fig. 3*b*).

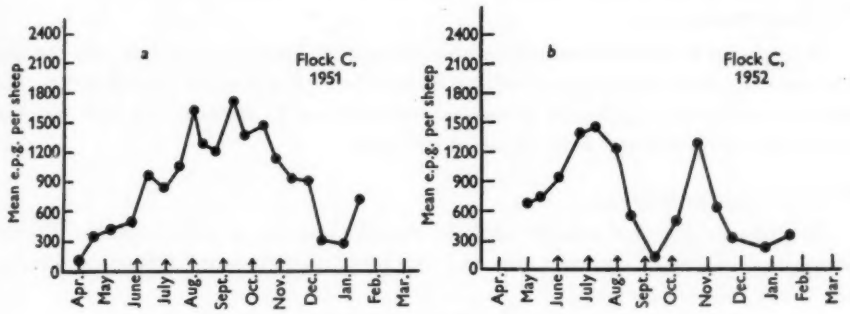

Fig. 3*a*, *b*. Worm egg counts. Flock C, 1951–2.

(*b*) *Seasonal incidence of different species*

Throughout the observations records were kept of the different species of worms represented by eggs in the faeces. Eggs of *Nematodirus* spp. and *Strongyloides* sp. were recorded at the time of counting. The samples were not examined for eggs of *Trichuris ovis*, records of this species being considered of insufficient interest to justify the extra time involved when a McMaster method of egg counting was being used. The data has been derived from differential larval counts and from the egg differentiation method described previously (Cunliffe & Crofton, 1953).

In the flocks considered, there appeared to be a regular pattern of succession of species which varied little from farm to farm and still less from year to year. While the timing appeared to be similar on each farm, the relative proportions of the different species differed not only from farm to farm but to a lesser extent from year to year. So far as could be determined, anthelmintic treatment of lambs in flocks B and C did not materially alter the pattern of succession, and the data available is insufficient to determine the effect of treatment on the relative proportions of the different species at different times of the year. The most striking difference between the Gloucestershire and Somerset farms was the difference in level of the *Nematodirus* infections. As in the ewes (see Crofton, 1954), the Gloucestershire figures for this species were much higher than the Somerset ones. To a lesser extent this was also true of *Strongyloides* but this will be discussed later.

Haemonchus contortus

As in the ewes this was present on all farms in very small numbers and was first detected by the egg differentiation method. Its presence was later confirmed by post-mortem examination. Never more than twenty worms were found and, from egg counts, the greatest numbers of this species appeared in early summer.

Ostertagia spp.

These worms formed the main bulk of the infection from the end of May until August. Small numbers of this species were found in April when the lambs were between 1 and 2 months old. In the latter part of the summer worms of this species were still numerous but less important numerically than *Trichostrongylus* spp.

Trichostrongylus axei

This species was found in small numbers from mid-April onwards but only became numerically important in August and September. For a short period during the time of maximum egg counts it was as numerous as *T. vitrinus*. In this way the lamb infections differed from those of the ewes.

Trichostrongylus vitrinus

The figures for this species include small numbers of other species *Trichostrongylus*, but as in the ewes this was the preponderant small intestinal form in the latter half of the year.

Cooperia spp.

These worms were most numerous in autumn and at this time were second only in importance to *Trichostrongylus*. There was some indication that *Cooperia* spp. were the most numerous species at the end of the winter—in February and early March, but the data is not sufficient to confirm this for all forms, because in many cases the lambs had been sold before this time.

Nematodirus spp.

The only species recorded was *N. filicollis*. This was the first species to establish itself in the lambs, and for a short period in April and May it was the most numerous species. It first appeared about the same time as *Moniezia*, and continued until autumn in small numbers. More detailed information about *Nematodirus* is available because the eggs are easily detected and recognized. Figs. 4–6 show the mean *Nematodirus* egg counts for flocks A–C respectively.

Except in the year 1950 the level of infection was higher on the Gloucestershire farms: flocks B and C, than on the Somerset farm: flock A. In flock A the maximum count for the 4 years occurred in 1950, there being nearly 250 e.p.g. at the end of June. In succeeding years the maximum counts were lower, although in 1953 and possibly 1952 the observations started too late for the main peak to be determined. In 1950 and 1951 the maximum numbers of eggs were recorded in late June. In 1952 the counts were low in June, the highest counts being recorded in May. In 1950–1 and 1953 counts remained below 50 e.p.g. after the end of July. In 1952 nearly 100 e.p.g. were recorded in August and in November there were more than 50 e.p.g.

In flock B, the maximum count in 1950 was about 150 e.p.g. In the remaining years the maximum counts were about 450 e.p.g. While the counts may not have started early enough in 1950 the apparent maximum occurred in June, this being the month of the maximum count in 1951. It is to be noted that there were more

than 250 e.p.g. from the end of April to the middle of July. In 1952 and 1953 maximum counts were recorded in May but in both years the period of high counts was spread over several months. In 1952 there were more than 250 e.p.g. from the end of April until the middle of August, while in 1953 counts of this magnitude were recorded from the end of April until mid-July. On one occasion after this time, at the beginning of September, there was another high count of more than

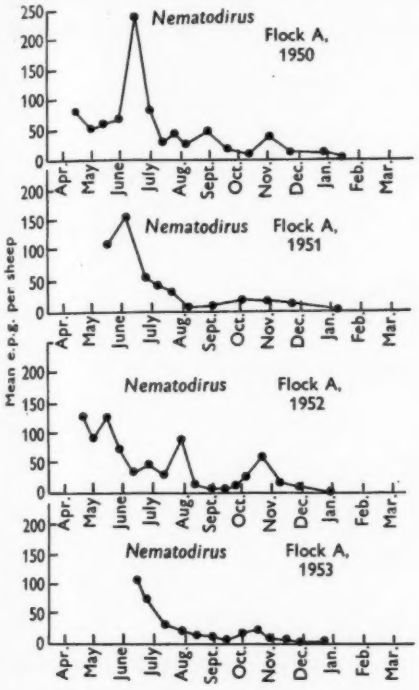
Nematodirus Flock A, 1950
Nematodirus Flock A, 1951
Nematodirus Flock A, 1952
Nematodirus Flock A, 1953
Mean e.p.g. per sheep
Apr. May June July Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar.

Fig. 4. Mean number of eggs of *Nematodirus*. Flock A, 1950–3.

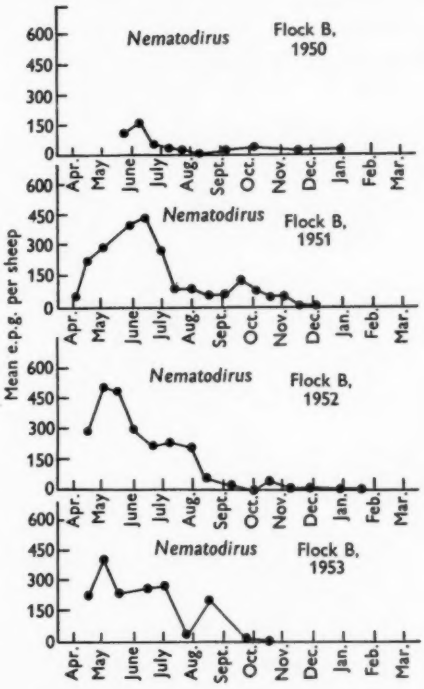
Nematodirus Flock B, 1950
Nematodirus Flock B, 1951
Nematodirus Flock B, 1952
Nematodirus Flock B, 1953
Mean e.p.g. per sheep
Apr. May June July Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar.

Fig. 5. Mean number of eggs of *Nematodirus*. Flock B, 1950–3.

150 e.p.g. In flock C, during the two years 1951 and 1952, the times of maximum counts was the same as in flock B, i.e. June in 1951 and May in 1952. In flock C, however, the peak was sharply defined in 1951, there being less than 150 e.p.g. before mid-June and after mid-July. In 1952, counts of more than 250 e.p.g. were recorded throughout the year from mid-May until mid-August, and even at the end of September there were 150 e.p.g. Not until mid-October were the counts less than 50 e.p.g.

Strongyloides papillosus

This species occurred sporadically throughout the year. While greatest numbers were recorded in the autumn, the incidence appeared to be more related to the type

of feeding of the lambs than to a seasonal factor. Increases occurred whenever lambs were fed on roots, rape or kale, and to a lesser extent when they were put on to stubble.

Other species

Bunostomum trigonocephalum; Oesophagostomum venulosum; Chabertia ovina and *Trichuris ovis* occurred in all three flocks, but the numbers were too low to show any very definite trends. *Bunostomum trigonocephalum* was found more frequently in summer, while *Chabertia ovina* and *Oesophagostomum* were more numerous in autumn and winter.

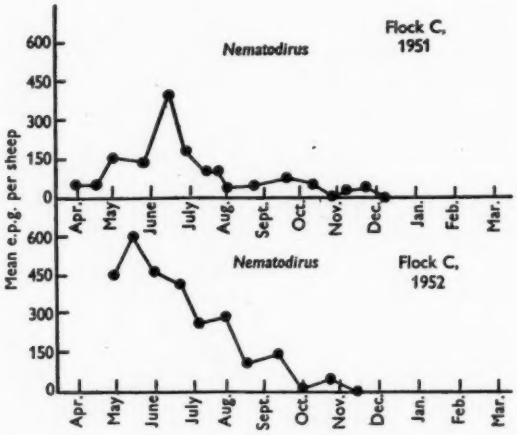

Fig. 6. Mean number of eggs of *Nematodirus*. Flock C, 1951–2.

(c) *Fluctuations in worm egg counts of individuals*

In a preceding section mean flock counts have been recorded. While this is convenient for the study of general trends, and is of importance when pasture contamination is to be considered, a more detailed study of the data is necessary for a better understanding of the dynamics of flock infection. Examination of egg counts for individual lambs has shown that there is considerable fluctuation from day to day, and even from one part of the day to another. Fig. 7*a–h* shows the counts for individual lambs plotted at 2- to 3-day intervals. These figures are based on samples taken at the same time of day on each occasion and are comparable in this respect. At first sight the fluctuations appear to be haphazard and the counts rising and falling in individuals without any apparent synchronization within the flock. Examination of Fig. 7*a–h* shows some indication of rhythmical fluctuations in the counts for each individual, and while the time of rise and fall of counts was not the same for different individuals, the general pattern of the rhythm was similar. This suggests that the fluctuations are not due to a particular or general environmental complex acting on the flock as a whole and that closer examination of the counts is desirable.

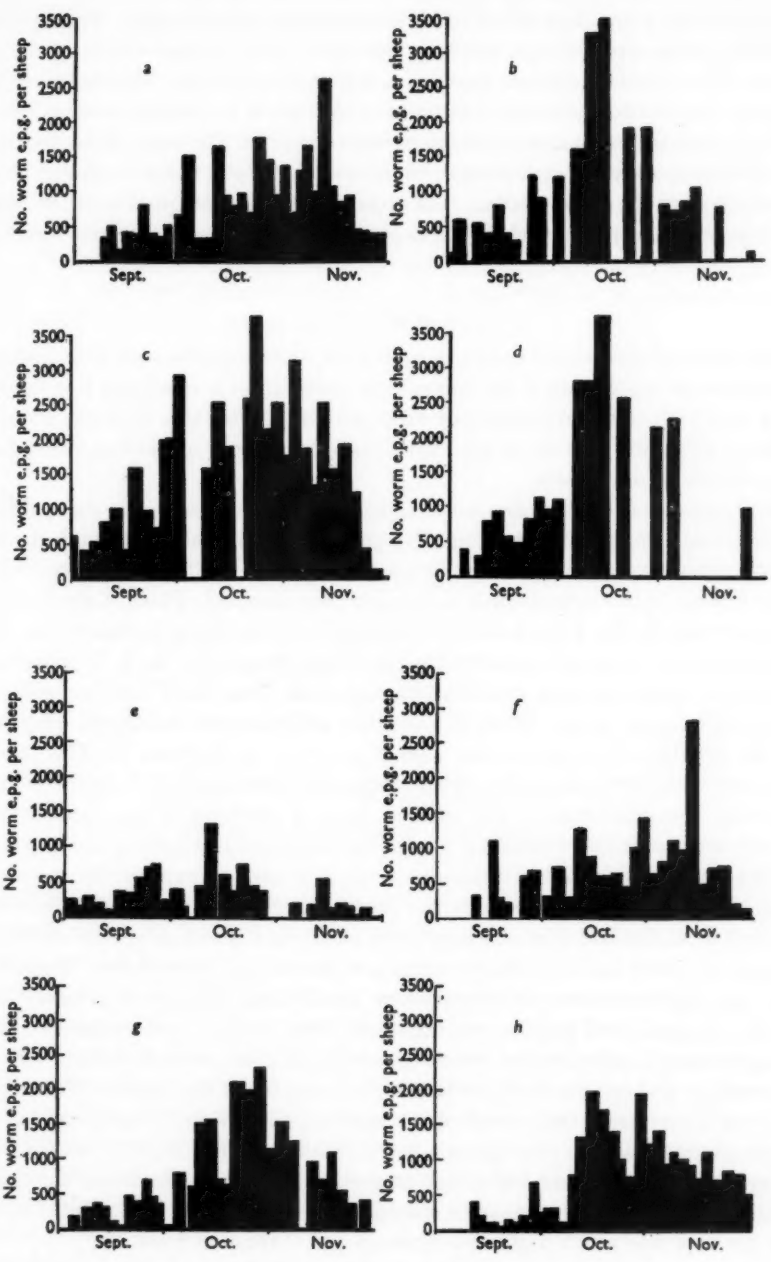

Fig. 7. *a–h*, worm egg counts of individual lambs.

The general pattern of egg counts in each case showed a period of rapid increase lasting a few days followed by a succession of low counts. The height of successive peaks produced in this way increased more or less regularly as time elapsed. The interval between peaks was relatively constant, varying from 2 to 3 weeks. In practically every case, after a high peak in autumn the counts fell rapidly and no further major fluctuations were observed. The time of the maximum peak differed in different individuals, but in the majority of cases maximum counts occurred within the same month. It is apparent that an integration of the individual results would produce the general picture presented in a previous section.

IV. DISCUSSION

In each series of observations, and in each flock, the most characteristic feature of the pattern of egg counts is the increase in count from a relatively low figure in spring to a high count in summer or early autumn. After this high egg count the reduction in counts is more or less rapid, there being only a small number of eggs in the winter in most cases.

The figures for each flock are not strictly comparable because the sheep differed in breed, management and stocking. The pastures differed also, although the main difference here lay in the more fertile pastures grazed by flock A. Moreover, the flocks differed in the anthelmintic treatment they received. Flock A received only one treatment in the 4 years under consideration, this being in September 1950. Flocks B and C received anthelmintic treatment frequently, flock B being dosed regularly at approximately monthly intervals from June until October with later treatment in some years. Flock C was given anthelmintic treatment irregularly, but the number of occasions was almost as great as for flock B. The times of treatment with either phenothiazine or copper-nicotine mixture is indicated in the diagrams. Consideration of the relative rates of stocking of the three farms is difficult because rates of stocking cannot be measured in terms of sheep per acre without giving a misleading impression; nor is it easy to express the stocking in terms of available nutritive material. Some impression of the position can be obtained from the fact that the number of lambs per acre was approximately the same on all three farms if the numbers per pasture are considered. On farm A, there was slightly more available pasture, facilitating changes of grazing. Additionally the quality of pasture was higher on farm A than on the other two farms. One important point which cannot be ignored is that the lambs in flock A were sold for slaughter and left the farm fat before the majority of the lambs in flocks B and C were sold as stores. Only some of the lambs in these latter flocks were fattened for slaughter. This not only gives some indication of the relative merits of the pastures and management but is also important because it illustrates the relative lengths of time for which the lambs were grazed on the farms. The number of lamb days per acre was much higher on farms B and C than on farm A.

When the observations started the flocks were fairly recently established. On farm B sheep were introduced in 1947, after a period of several years in which there had been no sheep. There was a history of parasitic disease in the ewes, there being

several deaths from this cause in early 1950. Flock A was introduced to their farm in 1949, and as far as can be ascertained there had been no sheep since about 1939. The general level of infection in the lambs over the period is of some interest. In flock A the maximum count recorded was about 2500, this occurring in 1950; thereafter the maximum for each year was lower, in 1951 it was 1800 e.p.g., in 1952 1100 e.p.g., and in 1953 100 e.p.g. In flock B the maximum count in 1950 was much lower than in flock A, but thereafter the maximum count increased each year until in 1953 it was 2200 e.p.g. This was despite anthelmintic treatment of lambs in flock B and none in the case of flock A (with the exception of September 1950). Flock C was observed for only 2 years, 1951 and 1952. The general pattern of infection was more like that in flock B than that in flock A. Regular anthelmintic treatment was given to the lambs in flock C.

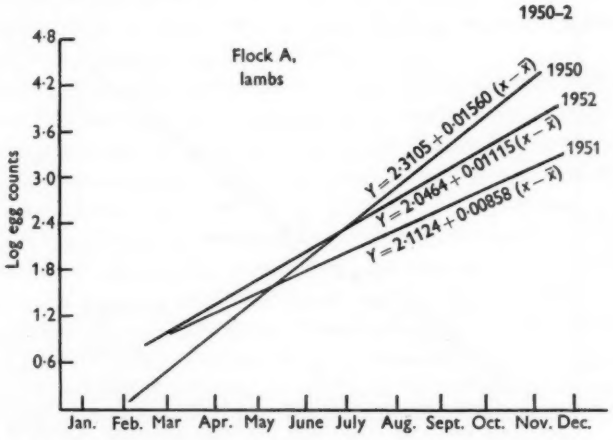

Fig. 8. Logarithm of egg count plotted against time in days (flock A).

One of the most striking features in all the graphs is the form of increase in egg count each year. At first the numbers increased slowly but the rate of increase became more rapid as the season advanced. In all cases, particularly in flock A, the rapid increase was followed by an even more marked decrease in counts. The form of increase appeared to be logarithmic and the counts were analysed to test this hypothesis. For flock A in 1950 the agreement between the observed figures and the curve plotted on the basis of logarithmic increase was very significant. (The probability of as good a fit occurring by chance being less than one in a hundred.) In succeeding years the agreement, though not quite so good, was still significant ($P = 0{\cdot}01$–$0{\cdot}05$) (Fig. 8). Even on farms B and C, where anthelmintic treatment had been given there was evidence of the form of increase being logarithmic ($P = 0{\cdot}05$–$0{\cdot}07$).

In 1950 two flying flocks of sheep adjacent to farm A were observed at irregular intervals. Although the number of observations was insufficient to give sufficient data for efficient analysis, the form of increase appeared to be again logarithmic.

While it is realized that no strict conclusions can be drawn from this insufficient data, and that logarithmic transformations can give spurious effects by their intrinsic nature, the logarithmic curve which fitted data for flock A was also a fairly good fit for the data obtained from these flying flocks D and E. Fig. 9 shows the curve calculated for flock A (1950) with points plotted for flocks A, D and E. It is interesting to note that in all cases the rapid reduction occurred at a time when another fortnight to 3 weeks increase at the previous rate would have resulted in counts of between 5000 and 10,000 e.p.g., counts which might be associated with clinical signs of disease. On the basis that the peak in egg counts would be reached 3 weeks before a count of 6000 e.p.g. would be expected, a calculation was made in 1952 to test the possibility of predicting the time of the peak. Using the data up to 14 July a prediction was made which was correct to within 2 weeks. In 1953 the

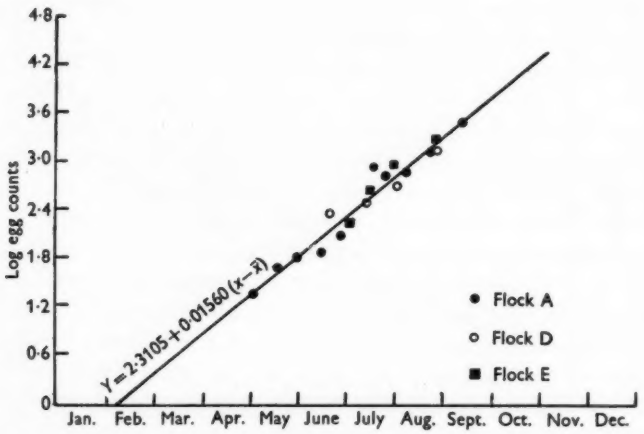

Fig. 9. Logarithm of egg counts plotted against time in days (flock A, 1950), with readings for flocks D and E plotted on the scale.

prediction was not so successful, being incorrect by 5 weeks. Consideration of the general form of the logarithmic curve suggests that any form of prediction from data early in the year must be subject to considerable error, and that the surprisingly accurate prediction in 1952 was fortuitous. Nevertheless, given sufficiently frequent sampling over a period not less than 12 weeks it would seem possible to predict the time of rapid increase, and therefore the danger period, some weeks before it occurred.

At this point it must be emphasized that climatic conditions might be expected to affect the rate of increase. In the area under consideration, however, the summer temperatures are sufficiently high to ensure rapid hatching of eggs and development of larvae; the humidity is sufficiently high and rainfall sufficiently frequent to prevent any approach to drought conditions. In areas of more extreme climate or in exceptional years the form of increase might not be expected to apply. This will be discussed later in this paper.

The implications of a logarithmic form of increase are of considerable importance. It implies, among other things, that the infection, at any time, as judged by egg counts, is proportional to a previous infection. That is, egg production is proportioned to previous pasture contamination by eggs passed by the same sheep. This can only be true under one of the following conditions:

(*a*) Any new infection replaces the previous one.

(*b*) Each new infection is added to the previous one and there is no interaction between old and new infections.

(*c*) A new infection modifies the rate of egg production of individual worms so that the total egg production is proportional to the number of newly developed worms and not to the newly developed worms plus the older worms. This might be explained on the basis of overcrowding, but even if it is accepted that this is possible, it must be remembered that there is no special reason why the increase should be logarithmic. If there were an overcrowding effect it might be expected to give a curve approaching the Pearl–Verhulst type.

(*d*) The rate of intake of infective stages which develop into adults is so large that the number of previous tenants is insignificant in comparison with the new infestation. This is a special case of that discussed in (*a*). Examination of the data shows that the rate of increase is not sufficiently great to justify this type of assumption.

While (*d*) seems to be least likely, it is difficult to obtain evidence to determine whether *a*, *b* or *c* are true. If either (*a*) or (*c*) were correct this would explain some of the difficulties of relating egg counts to worm populations in the host.

A further implication of the logarithmic curve is that pasture contamination must be proportional to the rate of egg production. This can be true only if: (1) the rate of development to the infective stage is relatively rapid, i.e. there is no prolonged delay in hatching or development; and (2) the larvae must have either a short life or a short period during which they remain infective.

As has been stated above, climatic conditions during the period of observation were such that (1) could be true. Previous work (Crofton, 1948; Taylor, 1938) suggests that (2) might also be true during the summer months.

Reference to the fluctuations of egg counts of individual lambs gives some indication that (*a*) above may be the correct explanation. If each new infection replaces the previous one then it might be expected that there would be a reduction in egg counts for a period between the loss of one infection and the end of the prepatent period of the new one. Moreover, this might be expected to be a cyclical phenomenon, if the developing larvae inhibited further establishment of new infections. Fig. 7*a–h* shows that periods of high counts and low counts alternate and that there is some constancy of interval between the peaks; that is, there are cyclical fluctuations and the basic period being 2–3 weeks. This period is within the time taken for development from infection to egg production, and therefore accords well with the suggested explanation.

On the basis of the logarithmic increase it is suggested that the worm population increases as a result of increasing 'doses' of infection, the stimulus of the host by the new infection being sufficient to cause elimination of the mature worms present. This is, in fact, postulating that the population increase is the resultant of a series

of 'self-cures' (see Gordon, 1948) occurring in an increasing series. As successive steps occur the stimulus becomes greater each time. In the case of the 'self-cures' described by Gordon the stimulus was usually supplied by a single dose of infective larvae. This dose was usually a massive one and frequently resulted in death as a result of the ensuing development of the larvae acting as a stimulus. (It is necessary to state here that Gordon (1948) suggested that the 'self-cure' phenomenon was not related to resistance of the host.) In the present case the gradually increasing dose might be presumed to result in an increase in level of resistance in the host. A 'self-cure' obviously occurs at the time of peak counts, the reduction in egg count being spectacularly rapid (see Fig. 1*a–d*). This must be an indication of the establishment of full resistance which prevents the 'stimulating dose' of infective larvae developing and establishing itself. The establishment of full resistance might be brought about by a different number of stages on different occasions there being numerous logarithmic patterns which might achieve the same end. Obviously any pattern of infection which did not produce a graded response might, if the infective dose was excessive, produce an initial self-cure followed by full development of the infective larvae constituting the stimulus. Under such circumstances the equilibrium between host and parasite would be destroyed and disease and possibly death might ensue.

Reduction in the rate of increase of the infecting dose might be expected to result in full resistance never being achieved. There is indication of this in the case of farm B, where presumably anthelmintic treatment has interfered with the rate of increase. The reduction of egg counts after the peak count was never as great or as rapid as on farm A, nor was the reduction always maintained.

This theory to account for the form of increase of populations in sheep would obviously account for the 'self-cure' phenomenon described by other workers and is analogous to the technique used in inducing immunity in the bacteriological field. It should be possible to verify this theory experimentally, although considerable care would have to be exercised in the gradation of the infective doses. The more general implications of the logarithmic increase are of considerable importance in practice, and some of these may be discussed now.

Throughout the period during which the logarithmic increase took place the composition of the infection changed. In the early part of the period the most important species was *Ostertagia*. At the time of the maximum count, species of *Trichostrongylus* were more numerous. There was, however, no apparent alteration in the form of increase, and it should be noted that at all times there were several species contributing to the total egg counts. It would seem, therefore, that the immunity built up in the lambs was not very specific, and that variation in the composition of species did not alter the general immune response. It may be objected that variation in egg production of differing species might cause considerable departures from the logarithmic curve, but it must be realized that, so far as could be detected from cultures and differential egg counts, the change in species composition was always gradual, and that, except in the case of *Nematodirus* spp. in the early part of the year, there were never fewer than three species present at any one time. *Haemonchus* being present in only very small numbers,

it is safe to assume that the change in species would not involve very great changes in the rate of egg production.

The straight-line regression between the logarithm of egg counts and time applies for the period during which observations were made each year. Extrapolation of the curve must be done cautiously however. Take for example the equation $Y = 2{\cdot}3105 + 0{\cdot}01560(x - \bar{x})$ calculated for the observations made in 1950 (Fig. 8). Substituting appropriate values we find that zero counts should have occurred on 6 February. This was before the main lambing time and it cannot be accepted that egg production started immediately after 6 February. It must be assumed that the regression line was not linear for the whole of the time between lambing time and the time of the first sample. This might be expected on other grounds. The infection is not self-inducing initially in so far as the initial infection comes directly from the pasture, the pasture itself being contaminated by the ewes and also possibly having some residual infection from the previous year. If this residual infection is small, and pasture counts suggest that this is so, then it follows that the initial infection of the lambs comes from the ewes and that the difference between the calculated time of initiation of infection in the lambs and the time of lambing is a measure of the amount of contamination of the pasture by the ewes. It is interesting to note that according to this argument the infection of lambs throughout the season is dependent upon the infection of the ewes in the early part of the year only. The spring rise in the ewes might govern the lamb infection by determining the level of initial infection. Certainly the main species contributing to the spring rise in the ewes is well represented in the early infections of the lambs but this similarity might arise as a result of a number of factors without there being a direct connexion between the ewe and lamb infections which has been suggested. If, however, the regression lines calculated for years 1950–2 for farm A (Fig. 7) are considered in relation to the height of the spring rise in figures given for ewes of this flock in a previous paper (see Crofton, 1954, fig. 1*a–d*) it will be seen that there is some evidence that the spring rise might be a major factor in initiating infection of the lambs. The only exception which must be made here is in the case of *Nematodirus* spp. These worms appeared almost simultaneously in the lambs and the ewes, and this suggests that infection of the lambs was initially a result of residual pasture infestation. Worms of the genus *Nematodirus* are exceptional in this respect, but this will be discussed in a later paper.

The constancy of pattern of the egg counts suggests a possible explanation of the results obtained in previous work on pasture infestation (Crofton, 1952). In this latter work it was shown that while the time of the maximum number of larvae on pastures varied from farm to farm and from season to season, the difference between farms was greater than the difference between seasons. It is obvious from the present work and from work on ewes described previously (1954) that the major contribution to pasture contamination must be made by lambs. The marked peak in egg counts might be expected to be reflected by a peak in the number of larvae recovered from the pastures. This has been confirmed by actual observations. In the present work the time of peaks of egg counts on the individual farms varied from season to season, and the results do not appear to support the sugges-

tion that the maximum time of pasture count varies little from year to year on the same farm. For various reasons, however, the time of lambing varied from year to year on the farms considered in this paper. It is suggested that a fixed time of lambing would tend to reduce the variation from year to year, and that differences in lambing time on different farms would account for differences in egg count peaks and, therefore, for differences in times of maximum recovery of larvae from pastures. That is, a fixed lambing time and constant stocking would give a relative constancy at the time of peak pasture infestation.

V. GENERAL CONCLUSIONS IN RELATION TO CONTROL MEASURES

Throughout the observations it must be noted that the lambs were not restricted to one pasture and that changes of pasture occurred fairly frequently, although rarely at intervals of less than 8 days. The change of pasture does not appear to have altered the pattern of increase, and it can only be concluded that contamination of a pasture is rapid, and that within broad limits the contamination of a pasture is a reflexion of the worm burden of the sheep grazing it. Movement of sheep from pasture to pasture cannot, therefore, be regarded as a method of control unless the intervals between moves are less than the time of development from egg to infective stages. This is probably less than 7 days in summer but rather longer in cooler times.

Movement of a flock at short intervals such as may occur in a close folding system would prevent the development of infection. As long as the parasite population is maintained at a low level this should be effective. The prevention of infection would also prevent the development of acquired immunity so that any breakdown in the system or any introduction of heavily infected animals would result in the exposure of susceptible animals to infections which they might not be able to withstand, and an outbreak of disease might occur. Outbreaks have been observed under these conditions.

The administration of anthelmintics does not appear to affect the general pattern of increase except perhaps to reduce its rate. The reduction in the rate of increase is obviously important, particularly in the production of fat lambs. There is good evidence from farm B, however, that immunity is not acquired to the same extent as in lambs which have remained undosed. With the cessation of anthelmintic treatment the egg counts tend to rise or at least do not fall as rapidly as in an undosed flock. Thus repeated dosing may reduce the total worm burden but acquisition of immunity may not be complete. This obviously has its dangers in certain circumstances.

In the areas under observation there were no droughts or other abnormal weather conditions which might delay hatching and development. Where such delays occur, and there is no accompanying high death-rate of the pasture contaminating stages, there is the possibility of enormous accumulations of infective stages which would become available when more normal climatic conditions reoccur. These accumulations would obviously cause a departure from the logarithmic form of increase, and might well produce an outbreak.

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HYPODERMA DIANA (DIPTERA, OESTRIDAE) AND *LIPOPTENA CERVI* (DIPTERA, HIPPOBOSCIDAE) AS PARASITES OF REINDEER (*RANGIFER TARANDUS*) IN SCOTLAND WITH NOTES ON THE SECOND-STAGE LARVA OF *HYPODERMA DIANA*

By D. S. KETTLE

Department of Zoology, West Mains Road, Edinburgh 9

and M. N. P. UTSI

Technical Adviser of the Reindeer Council of the U.K. and Managing Director of the Reindeer Co. Ltd.

The Reindeer Council of the United Kingdom was formed in June 1949 'to encourage experiments in reindeer-breeding in suitable areas in Scotland and/or overseas, and to engage in any appropriate activity ancillary thereto'. Two consignments have been shipped from Northern Sweden through Narvik to Scotland.

The first consignment, brought from Mr Utsi's own herd, comprised one 4-year-old draught ox, two 3-year-old bulls, two 2-year-old cows, and three 1-year-old female calves. These animals arrived in Scotland on 12 April 1952, and after being kept in quarantine for 6½ weeks* in the Edinburgh Zoo they were released on 27 May into a 300-acre area which had been fenced for the reindeer at Rothiemurchus, Inverness-shire (about 1000 ft. above sea-level).

The second consignment of ten reindeer, of which one bull and seven cows were forest reindeer, one a mountain reindeer cow, and one a cow of a third type, arrived at Grangemouth on 27 October 1952, and after 4 weeks' quarantine in Glasgow were released at Rothiemurchus on 25 November 1952. In addition, a mountain reindeer cow from Mr Utsi's Swedish herd, which had been 2 years in the London Zoo, and a zoo-born calf were added to the herd on 5 February 1953.

When a new species is introduced into a country, there is always the possibility that its parasites will spread to native species. This contingency can be guarded against by an adequate period of quarantine. Similarly, the introduced species becomes exposed to the possibility of infestation by local parasites. The imported reindeer have, in fact, become infested with the deer ked, *Lipoptena cervi*, and the deer warble fly, *Hypoderma diana*.

Hypoderma diana

In their native haunts, reindeer are infested with *H.* (*Oedemagena*) *tarandi* (Bergman, 1916; Hadwen & Palmer, 1922). To prevent the introduction of this parasite into the British Isles, the first consignment of reindeer was, in 1952, treated with derris root (in addition to hand extraction), as the Warble Fly

* The reindeer could not be moved after the designated 4 weeks' quarantine because of a local outbreak of foot-and-mouth disease. This long confinement, in Mr Utsi's opinion, had a very harmful effect on their condition.

(Dressing of Cattle) Order, 1948, was held to apply to them. In the spring of 1953 the Animal Health authorities agreed that hand extraction of larvae from the second consignment could be substituted for chemical control. The first consignment was then free from warbles. In both cases all larvae of *H. tarandi* were destroyed immediately after extraction.

In December 1953 the calf 'Sam', born at Rothiemurchus the previous May, was found to be infested with many warble larvae arranged along the spine under the skin. It became thin, weak, and unsteady on its hind legs, with gradually increasing posterior paralysis. On 14 December this calf died, and many larvae were found *in situ*. Three second-instar and 14 first-instar larvae were made available for identification. On 9 January 1954, the cow 'Ina' (of the second consignment) died after showing similar symptoms for several weeks, and at the post-mortem examination 9 living larvae were found under the skin, and of these 1 first-instar and 2 second-instar larvae were identified.

The behaviour of these larvae was abnormal for warbles as they made many holes in the skin and were relatively active, even attempting to leave the host in spite of the fact that they were only in the second instar. On 21 January 1954, the surviving reindeer were examined for warbles, and 18 second-instar larvae were removed from four reindeer while the others were free from warbles.

The larvae from 'Sam' and 'Ina', as well as those from live reindeer, were examined in detail, and compared with larvae occurring in red deer at Rothiemurchus at the same time,* and also with larvae obtained from red deer before the introduction of reindeer into Scotland. Details of this analysis are set out in Table 1. At this time we did not know how to differentiate between the second-instar larvae of *H. tarandi* and *H. diana* owing to the absence of an adequate description of *H. tarandi*. Bergman's account (1916) omits the detailed arrangement of the spiny bands.

On the ventral surface of a typical second-instar larva of *H. diana* there are anterior and posterior bands of spines on segments 2–7. In addition, there are antero-lateral patches of spines on segments 2–6, and postero-lateral patches of spines on segments 2–5. The dorsal surface, as is usual in larvae of the genus *Hypoderma*, is less spiny, and the posterior bands are undeveloped. There are paired patches of spines on each side of the midline in segments 2–3, and lateral patches in segments 2–4. This arrangement is in substantial agreement with that given by Brauer (1863), Cameron (1937) and Eichler (1941), for second-stage *H. diana* larvae. It is quite different from the arrangement in *H. tarandi*, where both anterior and posterior bands are well developed on all the ventral and nearly all the dorsal segments (see Table 1). Both Brauer (1863) and Eichler (1941) emphasize the absence or poor development of the anterior ventral band on the 7th segment. This did not apply to the material examined, except for collection 1 (Table 1). Cameron (1937) and Eichler (1941) limit the dorsal spines to the second and third segments, but our specimens agreed with Brauer's description in extending on to the fourth and very rarely on to the fifth segment.

* Through the kindness of Mr William Ironside, keeper of Lt.-Col. J. P. Grant's estate.

Eichler (1941) records *H. diana* as a parasite of the red deer, roe deer, fallow deer, elk and chamois. From this diversity of host it is perhaps not surprising that it should attempt to parasitize the reindeer when given an opportunity. Most of the red deer at Rothiemurchus leave the vicinity of the Reindeer Reserve* during the summer, and go higher up. These red deer have fewer warbles and some are even warble-free. However, some twenty red deer hinds are said to remain in the woods, and to be distinguished by being heavily parasitized by *H. diana*. It is probable that the reindeer acquired their infestation through the warbles left by these red deer near the Reserve in the spring.

It is known that some warble fly larvae undertake a complex migration through the body of their host to reach the final site of encystment on the back. This migration has been most fully worked out in the case of *H. lineatum* on cattle. Bishopp, Laake, Brundrett & Wells (1926) showed that *H. lineatum* proceeded by way of the oesophagus, while the larvae of *H. bovis* appeared to use the neural canal. Additional confirmatory data on *H. bovis* was supplied by Mote (1928). *H. bovis* larvae pass between the periosteum and dura mater, leaving behind a gelatinous streak. Cameron (1932) examined the gullets of twenty red deer, and failed to find any warble larvae, and he suggested that the larvae of *H. diana* might behave like those of *H. bovis*.

In this connexion, it is of interest to consider the post-mortem reports on the reindeer calf 'Sam' and the cow 'Ina'. In both cases the only unusual feature was the presence of a red gelatinous material in the epidural space, which appeared to be composed of blood plus cerebro-spinal fluid. In the absence of any other pathological changes, it seems reasonable to conclude that these two reindeer were killed as a result of damage to the central nervous system by migrating *H. diana* larvae.

As part of the morphological examination, a count was made of the number of chitinous rings in each of the posterior spiracles (Table 1). These data were subjected to an analysis of variance, and the results are presented in Table 2. For the analysis, the collections were placed in three groups:

(1) larvae recovered from red deer before the arrival of reindeer (collections 1–5);

(2) larvae collected from red deer in the vicinity of reindeer (collection 6);

(3) larvae collected from reindeer in the vicinity of red deer (collections 7 and 8).

It will be seen that there is no significant difference between these groups or between collections in the same group. The number of rings in each spiracle ranged from 12 to 27 with a mean of $18{\cdot}4 \pm 3{\cdot}5$.† In second-stage larvae of *H. tarandi* the corresponding range is 23 to 32 (Bergman, 1916), and in the eight larvae examined here 19 to 33 (Table 1) with a mean of $26{\cdot}7 \pm 3{\cdot}8$.†

It will be noted that there is significantly less variation between the two spiracles of an individual than between the spiracles of different individuals ($P_{\text{ind.}} < 0{\cdot}001$; r_1 (intraclass correlation) $= +0{\cdot}802$). This indicates that the number of rings in each hind spiracle of an individual is not independently determined.

* I.e. the original Reserve; from mid-December 1953, the reindeer have been grazed intermittently in a different area, at a height of 2000 ft. and more above sea-level.–M.U. April 1954.

† Mean ± standard deviation of population.

Table 1. *The frequency with which bands of spines occur on the various body segments and* diana *larvae collected from both red deer* (Cervus elap

(½ indicates that a lateral group of spines was present on only one side of a

Col. no.	Locality	Host	Date	No. of larvae	Anterior 2	3	4	5	6	7	8	9	10
1	Loch Rannoch	Red deer	Feb. 1937	9	9	9	9	9	9	3	—	—	—
2*	Ballachulish	Red deer	Jan. 1932	9	9	9	9	9	9	7	—	—	—
3	Either 1 or 2	Red deer	—	4	4	4	4	4	4	4	—	—	—
4†	Killin	Red deer	Feb. 1930	3	3	3	3	3	3	3	1	—	—
5†	Assynt	Red deer	1931	8	8	8	8	8	8	8	—	—	—
6	Rothiemurchus	Red deer	Jan. 1954	8	8	8	8	8	8	8	1	—	—
7	Rothiemurchus	Reindeer	Dec. 1953	5	5	5	5	5	5	5	1	—	—
		Sam, Ina	Jan. 1954										
8	Rothiemurchus	Reindeer	Jan. 1954	9	9	9	9	9	9	8	—	—	—
1–5	Not Rothiemurchus	Red deer	1930–37	33	33	33	33	33	33	25	1	—	—
6	Rothiemurchus	Red deer	1954	8	8	8	8	8	8	8	1	—	—
7, 8	Rothiemurchus	Reindeer	1953–54	14	14	14	14	14	14	13	1	—	—
9‡	*Hypoderma tarandi*	See footnote		8	8	8	8	8	8	8	8	8	8

Col. no.	Locality	Host	Date	No. of larvae	Anterior 2	3	4	5	6	7	8	9	10
1	Loch Rannoch	Red deer	Feb. 1937	9	7½	2	—	—	—	—	—	—	—
2*	Ballachulish	Red deer	Jan. 1932	9	9	6½	1½	—	—	—	—	—	—
3	Either 1 or 2	Red deer	—	4	4	3	—	—	—	—	—	—	—
4†	Killin	Red deer	Feb. 1930	3	2½	2½	1	—	—	—	—	—	—
5†	Assynt	Red deer	1931	8	7	6	—	—	—	—	—	—	—
6	Rothiemurchus	Red deer	Jan. 1954	8	6½	7	—	—	—	—	—	—	—
7	Rothiemurchus	Reindeer	Dec. 1953	5	3	1	—	—	—	—	—	—	—
		Sam, Ina	Jan. 1954										
8	Rothiemurchus	Reindeer	Jan. 1954	9	9	7½	1½	—	—	—	—	—	—
1–5	Not Rothiemurchus	Red deer	1930–37	33	30	20	2½	—	—	—	—	—	—
6	Rothiemurchus	Red deer	1954	8	6½	7	—	—	—	—	—	—	—
7, 8	Rothiemurchus	Reindeer	1953–54	14	12	8½	1½	—	—	—	—	—	—
9‡	*Hypoderma tarandi*	See footnote		8	8	8	8	8	8	8	8	7	2

* One specimen without posterior spiracles.

† Specimens loaned by Mr J. W. McHardy.

‡ Two specimens loaned by British Museum (Natural History), which had no data; and six specime from Caribou in 1948.

the various body segments and the number of rings in the posterior s
from both red deer (Cervus elaphus) *and reindeer* (Rangifer tarandus

was present on only one side of a larva. For comparison the same data are

Ventral

Anterior							Posterior								
4	5	6	7	8	9	10	2	3	4	5	6	7	8	9	10
9	9	9	3	—	—	—	9	9	9	9	9	9	—	—	—
9	9	9	7	—	—	—	9	9	9	9	9	8	1	—	—
4	4	4	4	—	—	—	4	4	4	4	4	4	—	—	—
3	3	3	3	1	—	—	3	3	3	3	3	3	—	—	—
8	8	8	8	—	—	—	8	8	8	8	8	8	—	—	—
8	8	8	8	1	—	—	8	8	8	8	8	8	—	—	—
5	5	5	5	1	—	—	5	5	5	5	5	5	1	—	—
9	9	9	8	—	—	—	9	9	9	9	9	9	1	—	—
Summary															
33	33	33	25	1	—	—	33	33	33	33	33	32	1	—	—
8	8	8	8	1	—	—	8	8	8	8	8	8	—	—	—
14	14	14	13	1	—	—	14	14	14	14	14	14	2	—	—
8	8	8	8	8	8	8	4	8	8	8	8	8	8	8	8

Dorsal

Anterior							Posterior								
4	5	6	7	8	9	10	2	3	4	5	6	7	8	9	10
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1½	1½	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1½	1	—	—	—	—	—	—	1	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	½	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1½	1½	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Summary															
2½	2½	—	—	—	—	—	—	1½	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1½	1½	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8	8	8	8	8	8	7	2	1	4	8	8	8	8	8	8

hich had no data; and six specimens loaned from the Department of Agricu

posterior spiracles of second-instar Hypoderma
er tarandus)

me data are given for *H. tarandi*.)

			Antero-lateral						Postero-lateral				
8	9	10	2	3	4	5	6	7	2	3	4	5	6
-	—	—	9	9	9	7½	4	—	4	7	7½	4½	—
1	—	—	9	9	9	9	8	—	5	5½	8	7	—
-	—	—	4	4	4	4	2½	—	2½	3	3	2	—
-	—	—	3	3	3	3	2½	1	3	2½	2	2	1
-	—	—	7	8	8	8	8	½	3½	6	5½	6	—
-	—	—	8	8	8	8	8	—	5	6	6	5½	—
1	—	—	5	5	5	4½	4	—	1	3	2½	2	—
1	—	—	9	9	9	9	6	—	3	7	6½	2	—
1	—	—	32	33	33	31½	25	1½	18	24	26	21½	1
-	—	—	8	8	8	8	8	—	5	6	6	5½	—
2	—	—	14	14	14	13½	10	—	4	10	9	4	—
8	8	8	Not observed						Not observed				

			Antero-lateral				Posterior spiracles Number of chitinized rings	
8	9	10	2	3	4	5	Range	Mean ± S.D.
-	—	—	8	8½	3	—	12–27	19·3 ± 4·1
-	—	—	9	9	5½	—	16–24	20·1 ± 2·8
-	—	—	4	4	3½	—	13–21	16·9 ± 2·7
-	—	—	3	3	2	1	14–24	18·2 ± 3·6
-	—	—	8	8	7	—	15–25	18·8 ± 2·8
-	—	—	8	8	5	—	12–23	16·9 ± 3·3
-	—	—	5	5	3½	—	14–27	20·5 ± 3·5
-	—	—	9	9	5	—	14–23	16·4 ± 2·3
-	—	—	32	32½	31	1	12–27	19·0 ± 3·4
-	—	—	8	8	5	—	12–23	16·9 ± 3·3
-	—	—	14	14	8½	—	14–27	17·9 ± 3·4
8	8	8	Not observed				19–33	26·1 ± 3·4

t of Agriculture, Ottawa, Canada. The latter were obtained

Lipoptena cervi

On 20 December 1952, two of the mountain reindeer of the first consignment (a 3½-year-old bull and 1½-year-old cow) were seen to have become infested with *L. cervi*. The bull was infested with many keds, but the cow had only a few; the other reindeer in the herd were ked-free. During January the bull showed signs of pining and it was last seen on 26 February 1953. Later the cow started pining and it died on 11 May 1953. The material forwarded for identification consisted of 18 females, 13 males and 8 puparia of *L. cervi*. (All keds found were removed on 20 December, and Gammexane Byre Spray rubbed on the reindeer.)

Table 2. *Analysis of variance of number of chitinous rings in posterior spiracles of second-instar* Hypoderma diana *larvae*

(Collections 1–8 in Table 1 have been grouped into: (1) red deer before arrival of reindeer (cols. 1–5), (2) red deer in vicinity of reindeer (col. 6) and (3) reindeer in vicinity of red deer (cols. 7 and 8).

Source	Sum of squares	Degrees of freedom	Mean square
Between groups	63·3	2	31·65
Between collections	169·0	5	33·8
Within individuals	98·5	54	1·82
Random error	761·2	46	16·54
$F_{col.} = 2{\cdot}04$	$F_{gp.} = 1{\cdot}91$	$F_{ind.} = 9{\cdot}09$	
$P_{col.} = 0{\cdot}1$	$P_{gp.} = 0{\cdot}15$	$P_{ind.} < 0{\cdot}001$	

The F test has been applied to determine whether the between groups and the between collections variance is larger and the within individuals variance smaller than the random error.

In September 1953, seven of the reindeer developed light infestations of *L. cervi*. Ninety-four specimens were collected for identification. Of these, half were sent to Dr J. Bequaert of the Museum of Comparative Zoology, Harvard, U.S.A., who confirmed the identification. Most of these keds were winged, or had but newly shed their wings, and the forty-seven specimens in our possession consisted of 20 females and 27 males. By October 1953, only a few keds were to be found on the reindeer; these were removed, and by the middle of January 1954 none could be found.

Mr Utsi has never seen keds on reindeer in Northern Scandinavia, and Hadwen & Palmer (1922) make no mention of pupiparous parasites on reindeer in Alaska. Bequaert (1942) records *L. cervi* from the roe deer, red deer, elk and sika deer, and believes that it was introduced into North America about the end of the nineteenth century, and that it has spread to the native Virginia deer (*Odocoileus virginianus*). In a letter Dr Bequaert suggests that the absence of *Lipoptena cervi* on reindeer in their native haunts may be due to the fact that no species of *Lipoptena* is known to occur north of 60° N.

The area of the Reindeer Reserve had been occupied by red deer in force until April 1952, when the fence was finally completed. In his book on the red deer, Fraser Darling (1937) states that the puparia are shed with the hair in April,

remaining dormant on the ground until the emergence of the adults in September. This would explain the development of an infestation on the reindeer in the autumn of 1952. However, he has never found puparia on red deer in winter, but only after the end of March (p. 143). On the reindeer, puparia were present in December. This change in behaviour may be a reaction to a new host. The winged keds, which were found on the reindeer in September 1953, are unlikely to have originated from the earlier infestation, as the heavily infested beast was probably poached at the end of February, and the lightly infested beast alone survived until 11 May 1953.

SUMMARY

Some of the reindeer (*Rangifer tarandus*) introduced into Scotland from Northern Sweden have become infested with the deer warble fly *Hypoderma diana* and the deer ked *Lipoptena cervi*.

Evidence is presented which suggests that the larvae of *H. diana* proceed to their final site on the back of the host by way of the neural canal, and in this way probably caused the deaths of two reindeer in the winter of 1953–4.

A detailed analysis is presented of the frequency with which various bands of spines occurred on second-stage larvae of *H. diana*. An analysis of the number of chitinous rings in the two posterior spiracles on second-stage *H. diana* larvae showed that in any individual larva the two numbers were not independently determined.

On reindeer *L. cervi* produced puparia in December.

We wish to record our thanks to Dr J. C. Bequaert, Harvard, U.S.A., for confirming the identification of *L. cervi*; to Messrs G. A. M. Sharman and T. G. Marr of the North of Scotland College of Agriculture County Advisory Service, Inverness-shire, for making available their post-mortem reports; to Mr J. W. McHardy, of the Department of Agriculture and Forest Entomology, University of Edinburgh, for the loan of collections 4 and 5 (Table 1); to Mr H. Oldroyd of the British Museum (Natural History) and to Mr G. E. Shewell of the Canadian Department of Agriculture, Ottawa, for the loan of specimens of *H. tarandi*.

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ON TWO NEW SPECIES AND NEW RECORDS OF ACARINA MAINLY FROM THE ETHIOPIAN REGION

By M. M. J. LAVOIPIERRE

Department of Entomology and Parasitology, Liverpool School of Tropical Medicine

(With 9 Figures in the Text)

In this paper two new species of Acarina are described, the first a new species of *Pterygosoma* from a West African agamid lizard and the second a new species of *Schöngastiella* from two West African rodents. In addition, several new records of Acarina are given, most of these from the Ethiopian region of Africa. The writer wishes to extend his thanks to all those who have contributed material, and in particular Mr W. Crewe and Dr R. Taufflieb.

ACARINA

Pterygosomidae

The scale mites of lizards have been the subject of several studies, the most extensive of which are those of Lawrence (1935, 1936) on the South African species. The species of *Pterygosoma* described below seems, however, to be the first species of this genus to be recorded from West Africa and the specimens from which it is described were collected in Liverpool from an agamid lizard, *Agama agama*, which had recently been sent to the Department of Zoology, of the University of Liverpool, from the Loiasis Research Centre at Kumba in the British Cameroons.

Living specimens and specimens preserved in alcohol were obtained for study and were all mounted in Berlese's gum chloral mountant after being cleared in lacto-chloro-phenol. In several of the specimens, which were mounted directly in the gum choral mountant, the respiratory system was clearly visible after the mites had been left standing for a few days. The writer has used this procedure with other acarines, notably with *Syringophilus columbae* Hirst, and has obtained successful results, though such preparations are unfortunately seldom permanent and require immediate study. The respiratory system of the genus *Pterygosoma*, as represented by the new species, shows two short main trunks which divide into several branches. The respiratory system of the pterygosomids does not appear to have been observed previously.

The new species is named in honour of Mr W. Crewe, of the Loiasis Research Scheme, Kumba, who drew the attention of the writer to the presence of these acarines on the lizards kept in captivity in Liverpool.

Pterygosoma crewei n.sp.

Type female (Fig. 1) from *Agama agama*. Colour in life: bright vermilion. General appearance: shape similar to other species of the genus *Pterygosoma*, particularly to the *hispida* group as defined by Lawrence. The new species is closely allied to *P. agamae* Peters, recorded from Tanganyika, by Peters in 1849 and from South Africa by Lawrence in 1936. The mouthparts are short and stout but have no characters of taxonomic significance. Dorsal surface: along the anterior shoulders is the usual series of spiculate hairs. Two pairs of spiculate hairs, slightly thickened terminally are distinctly set away from the shoulder hairs; the second pair not spatulated, as in *P. agamae*.

In the figure of *P. agamae* given by Hirst (1926) there are 34 peripheral hairs on each side, whereas in *P. crewei* there may be any number between 19 and 27 (the type possesses 23). Lawrence (1935, 1936) has drawn attention to the considerable variation that may exist in the numbers of these hairs. In *P. crewei* n.sp. the peripheral hairs are slightly expanded terminally and finely spiculated. Hirst does not show spiculation of these hairs in his figure of *P. agamae* and makes no mention of this character in his description. In *P. agamae* one of the peripheral hairs is clearly stouter and larger than the others, a character which is absent from the West African species.

Anal field (Fig. 3). Six pairs of hairs flank the anal opening. These hairs were difficult to see from the dorsal surface so that the anal area had to be dissected free of the body. It is essential that this dissection should be undertaken where possible as the morphology and arrangement of these hairs is important taxonomically. The anal hairs in *P. crewei* n.sp. are arranged in two groups; an anterior group of two pairs and a posterior group of four pairs. In *P. agamae* the first pair of anal hairs is markedly expanded terminally, thus differing considerably from *P. crewei* n.sp. In some of the specimens examined there were only five hairs present on one side of the anal opening and six on the other side; there therefore appears to be a certain amount of variation in this character. All the hairs are stout, with striations and terminally, coarsely spiculated. Flanking the anal opening there are, anteriorly, two pairs of small spines, and, posteriorly, one pair of much stouter spines.

Measurements. Length: 0·46–0·63 mm. (12 specimens): 9 of these measured between 0·50 and 0·55 mm. Breadth: 0·87 mm. (12 specimens). The breadth appears to be very variable.

Male (Fig. 2). The male of this species is quite unlike those described for *P. hystrix*, Lawrence and *P. bicolor* Lawrence. In these two species the penis is directed posteriorly; in the new species the penis is directly anteriorly. Both Lawrence's species were described from gerrhosaurid lizards and he gave no descriptions of any male pterygosomid mites from agamid lizards. It seems probable, however, that the males of other species of pterygosomid mites will be found to have a forwardly projecting penis, as is the case in *P. crewei* n.sp. If this proves to be the case then there seems little doubt that the genus *Pterygosoma* will have to comprise two subgenera, as has already been suggested by Lawrence.

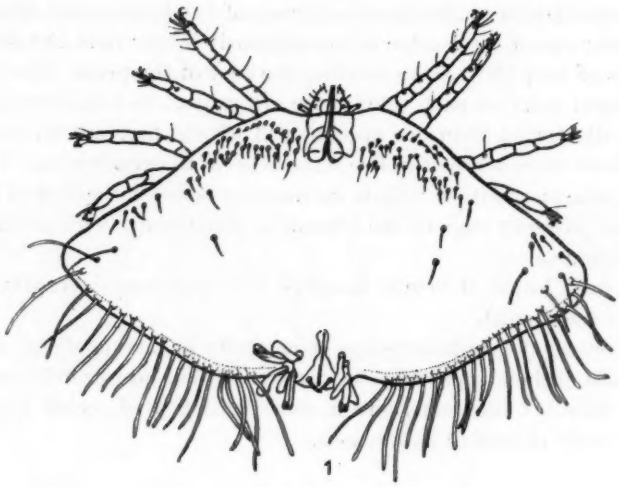


Fig. 1. Dorsal view of the female of *P. crewei* n.sp.

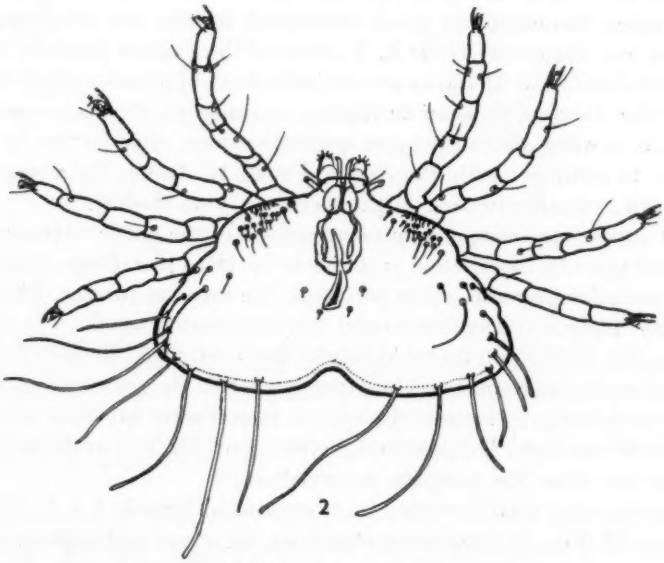


Fig. 2. Dorsal view of the male of *P. crewei* n.sp.

General appearance: heart-shaped and considerably smaller than the female. Dorsal surface: there are approximately twenty-five shoulder hairs on each side. Posterior to the shoulder hairs there is a group of five longer setae which are very finely serrated; one of these setae is conspicuously longer than the others. There are two pairs of very short setae flanking the root of the penis. There are five to seven peripheral hairs on each side; these are subject to considerable variation. The male is illustrated from one specimen in which there are six on each side. Ventral surface: there is a pair of fine setae close to the gnathosoma. Two pairs of longer setae arise at about two-thirds the distance between the base of the gnathosoma and the posterior edge of the idiosoma. Mouthparts with no characters of taxonomic significance.

Measurements: length 0·26 mm. (average of 4 specimens); breadth: 0·48 mm. (average of 4 specimens).

Notes. About sixty lizards were kept in captivity in Liverpool and, according to Mr Crewe, most of these appeared to be parasitized. The host lizard is very common at Kumba, British Cameroons, and is semi-domesticated, being found sunning itself on the walls of human habitations.

TROMBICULIDAE

Schöngastiella tauffliebi n.sp.

A distinctive species easily differentiated from the other species of this genus described from Central Africa by the setal formula of the coxa: 1.1.4. It also possesses other characteristics which distinguish it from the other species. The new species was discovered by Dr R. Taufflieb of the Pasteur Institute of Brazzaville, French Equatorial Africa, on several individuals of a rodent which is common at Brazzaville, *Praomys jacksoni* de Winton and once on *Mastomys coucha* Smith. Dr Taufflieb, in whose honour the new species is named, remarks that he has often collected it in company with *Trombicula giroudi* M. André, but notes that it is always found in small numbers. Eight specimens were studied.

General appearance: shape as in other species of the genus *Schöngastiella* but there was no sign of a constriction posterior to the third pair of legs; this may have been due to undue pressure of the coverslip. No eyes are present. There are approximately thirty-two dorsal setae and forty-six ventral setae.

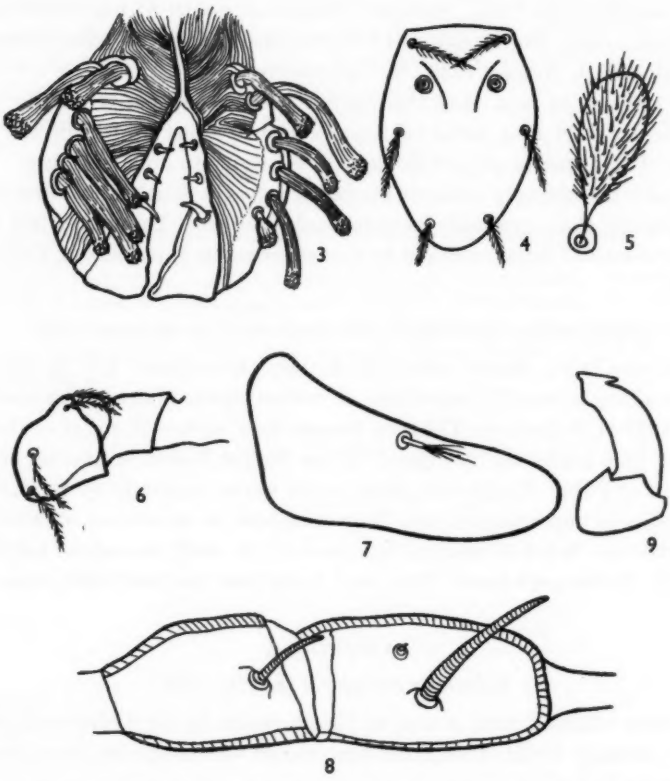
Scutum (Fig. 4): the scutum could only be made out with difficulty in one specimen and in another specimen was only partly visible. The scutal setae are all very similar in morphology. The second posterior lateral setae are very much shorter than the other two pairs. The pseudostigmatic organ (Fig. 5) is ovate and is covered with many fine setae; the peduncle is very short.

Legs: trochanteric setal formula 1.1.1. coxal setal formula 1.1.4. (Fig. 6). The seta on coxa II (Fig. 7) differs somewhat from the others and appears to be comprised of a short stalk from which arise barbs of different lengths. The setae on coxa II measures 25 μ. The spur of tarsus 1 (Fig. 8) in nearly all the specimens is slightly curved, particularly at the base and is long.

Mouthparts: the dorsal setae of segments II, III and IV of the palp are barbed,

thus differing from the other known species which have no barbs. The palpal tarsus bears a prominent barbed seta terminally. The chelicera is short and stout (Fig. 9).

The standard measurements are as follows: LC 345μ (5 specimens); IC 261μ (5 specimens); pa 159μ (6 specimens); pm 132μ (6 specimens); pp 153μ (6 specimens); LGn 57μ (7 specimens); IGn 66μ (7 specimens); LPn 36μ (7 specimens);



Figs. 3–9. Fig. 3. The anal area of the female of *Pterygosoma crewei* n.sp. showing the anal hairs and the spines flanking the anal opening. Fig. 4. The scutum of *Schöngastiella tauffliebi* n.sp. Note that the pseudostigmatic organs are not shown in the drawing; they were absent in the specimen from which the scutum was drawn. Fig. 5. The pseudostigmatic organ of *S. tauffliebi* n.sp. Fig. 6. Left coxa III of *S. tauffliebi* n.sp., showing the four coxal setae. Fig. 7. Left coxa II showing the morphology of the coxal seta of *S. tauffliebi* n.sp. Fig. 8. Left tarsus I and tibia I of *S. tauffliebi* showing the tarsal spur. Fig. 9. Chelicera of *S. tauffliebi* showing the tarsal spur.

LE 78μ (1 specimen); IEA 40μ (1 specimen); IES 50μ (1 specimen); IEP 57μ (1 specimen); IEP′ 30μ (1 specimen); AW 30μ (7 specimens); PW 36μ (7 specimens); P′W′ 15μ (7 specimens); SB 21μ (7 specimens); AP 30μ (7 specimens); AP′ 57μ (7 specimens); AL 21μ (7 specimens); P 30μ (7 specimens); P′ 21μ (7 specimens); S 27μ (1 specimen); LpS 4μ (1 specimen); LmS 23μ (1 specimen); ImS 10μ (1 specimen); H 23μ (7 specimens); V 17μ (7 specimens); D 29μ (7 specimens).

This species is characterized by the setal coxal formula, the morphology of the seta of coxa II, the character of the dorsal setae of the palp and the curvature of the spur of Tarsus I.

DERMANYSSIDAE

Bdellonyssus bacoti (Hirst, 1914)

Eleven females from 'rats', Schagen, Transvaal, South Africa, 20 October 1940 (collector unknown). One female and five nymphs from *Rattus rattus*, Pietermaritzburg, South Africa, August 1936 (M. Lavoipierre).

It is of interest to note that this species breeds very easily on the young of *Mastomys coucha*. A long series of females and nymphs were collected from an individual of this species of gerbille kept in captivity in Johannesburg.

This species is extremely common on *Rattus rattus* in South Africa and occurs far more commonly than available records would indicate. The writer has time and time again identified large numbers from rats, from the Provinces of Natal and the Transvaal.

Hirstionyssus latiscutatus (de Meillon & Lavoipierre) 1944

Nine females from *Rattus rattus*, Holfontein, Kroonstad, O.F.S., 14 January 1939. Also a long series of females from *R. rattus* collected between 18 January and 27 March 1939 at Holfontein. Thirteen females from nest of *R. rattus* at Holfontein, 27 October 1939 (collected by officials of the Plague Research Station of the S.A. Department of Public Health and given to the writer for study by D. H. S. Davis).

This species is appearing in such large numbers in collections of Acarina from *R. rattus* that one is led to believe that some of the early records of infestation of premises by *Bdellonysus bacoti* Hirst may have been confused with this mite.

LAELAPTIDAE

Eulaelaps stabularis (Koch) 1839

Six females collected from a nest of *Tatera indica* by Dr Delpy near Hessarek, Persia (20 January 1943). This is the first record of this species from Persia.

Hypoaspis (*Androlaelaps*) *theseus* Zumpt 1950

Six females collected in the Southern Transvaal on *Tatera brantsii* on 28 August 1943. Four of these females have the posterior edge of the sternal plate very coarsely denticulated. Zumpt does not draw attention to this variation in his description of this species.

Macrolaelaps muricola (Tragardh) 1908

From Gerbille nests, Theodora Farm, Weiveld Siding, Orange Free State, South Africa, December 1925. Gerbille nests, Barrage, Vaal River, Orange Free State, South Africa, April 1925. Also from a mixed lot of ectoparasites collected from two rodents, *Rattus* (*praomys*) *tullbergi* and *Lophuromys sikapusi* caught in the forest of Llaro, near Abeokula, Southern Nigeria (L. Bruce-Chwatt).

MACROCHELIDAE

Macropheles muscae (Scopoli) 1772

Thirteen females from *Musca* sp., Dombashawa Agricultural School, near Salisbury, Southern Rhodesia, November 1939. These specimens were sent to the writer by Alexander Cuthbertson. The species has a very definite controlling effect on the housefly, *Musca domestica* and is highly beneficial. All but the first instar (proteronymph) preys on housefly eggs. It has been the subject of a detailed study by C. Pereira and M. P. de Castro (1945) in Brazil.

This is a new record for South Africa.

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IXODES HOOGSTRAALI, A NEW SPECIES OF TICK FROM YEMEN

By DON R. ARTHUR

Zoology Department, King's College, University of London

(With 11 Figures in the Text)

In January 1954 I received from Dr Harry Hoogstraal of the United States Naval Medical Research Unit no. 3, Cairo, Egypt, a collection of ticks for investigation. In the monograph of Nuttall, Warburton, Cooper & Robinson (1911) these specimens were run down to *Ixodes ugandanus* Neumann, but in view of the constant differences between them I recognize the present material as a new species. I assign the name *hoogstraali* to them in consideration of Harry Hoogstraal's kindness and generosity in supplying me with tick material. Unfortunately the entire collection consists of females.

Holotype. Female from *Meriones rex buryi* (HH 6354–56), 6 miles south of Ma'bar (alt. 7400 ft.), Yemen, 6 February 1951. H. Hoogstraal legit. Deposited in United States National Museum.

Paratypes. Twenty-one females, all from same host as holotype. Two paratypes deposited in each of the following: Research Station, Onderstepoort (Dr G. Theiler); Rocky Mountain Laboratory, Montana, U.S.A. (Mr Glen M. Kohls); British Museum of Natural History (Dr G. Owen Evans) and in the private collection of Dr H. Hoogstraal, United States Naval Medical Research Unit no. 3, c/o American Embassy, Cairo, Egypt.

DESCRIPTION

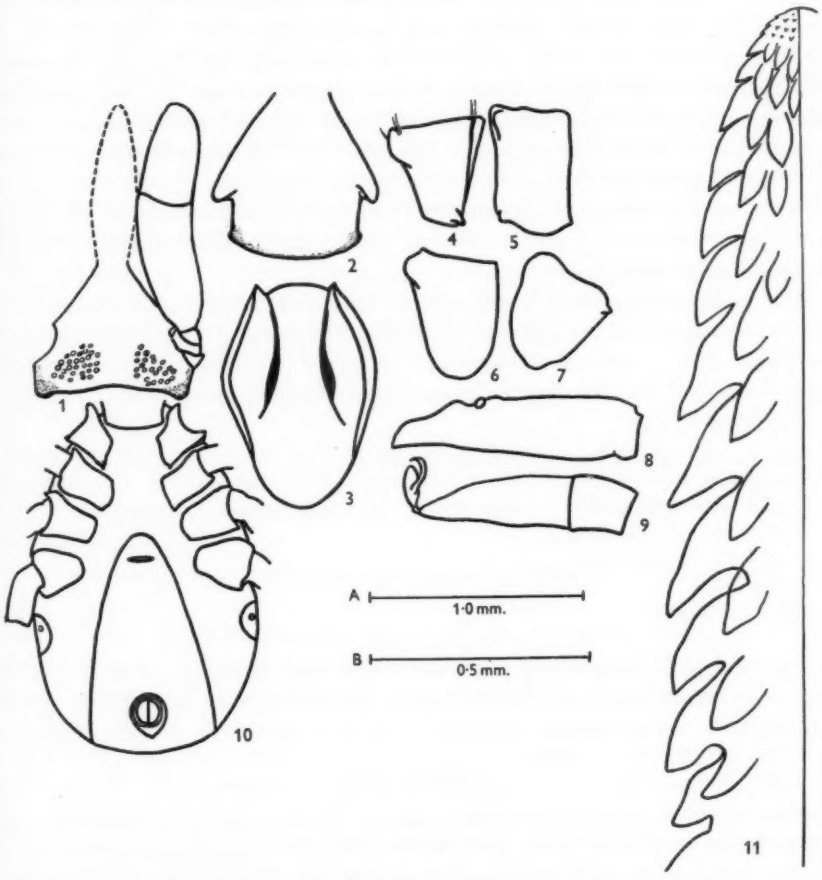
Female. Body colour pale brown, scutum, legs and capitulum of darker colour. Unfed females measure 2·3 mm. Gorged females measure 5·2 mm.

Capitulum (Fig. 1). Length 0·61 mm., width of basis along dorsal ridge 0·34 mm. Postero-lateral margins short, divergent, straight or a little curved. Posterior margin straight or only very slightly concave, cornuae (*if present*) small, broad protuberances: postero-lateral angle more heavily pigmented. Surface mildly convex. faintly crazed, impunctate and without hairs. Porose areas separated by about the distance of their greatest width, subtriangular, superficial with the pores well separated from one another (Fig. 1). Palpi long, concave on outer margin, correspondingly convex on mesial surface. Base of article II closely pressed to side of basis, widest at middle of combined length of articles II and III. Combined length of articles II and III about 0·52 mm., greatest width 0·10 mm.

In ventral view (Fig. 2) basis narrows in front of posterior margin, postero-lateral angles rounded and posterior edge broadly rounded and salient, heavily pigmented. No transverse suture evident. Greatest width across the auriculae. Auriculae as broad retrograde spurs directed backwards and downwards; surface crazed, smooth and shining. Inner surfaces of palpi asymmetrically concave,

Palpal article I broader than long, with a distinct forwardly directed plate-like extension arising from it and bearing one long, stout hair.

Hypostome (Fig. 11). Long and moderately broad, profile very slightly curved, tip rounded. Denticles from base to apex arranged as 6 rows of 2/2 files, 3–4 rows of 3/3 files and 1–3 rows with 4/4 files; a small tooth-bearing corona present. Median triangular unarmed area. Length about 0·38 mm.



Figs. 1–11. *Ixodes hoogstraali* female. Fig. 1. Capitulum, dorsal view. Fig. 2. Basis capituli, ventral view. Fig. 3. Scutum. Figs. 4–7. Coxae I–IV. Fig. 8. Tarsus I. Fig. 9. Tarsus IV. Fig. 10. Ventral view of the opisthosoma showing the position of the genital orifice and of the closed pointed anal groove. Fig. 11. Hypostome. (Figs. 1–9, scale B; Fig. 10, scale A.)

Scutum (Fig. 3). Length 1·01 mm., breadth 0·73 mm. Lozenge-shaped with greatest breadth in front of the middle, narrowing posteriorly and becoming strongly rounded. Length : breadth as 1·4 : 1·0. Surface finely punctate, few short white hairs in the anterior portion. This is in contrast to the alloscutum where long, white, curved hairs are numerous. Cervical grooves long, converging at first and then diverging but not extending laterally to the scutal margins. The cervical

depression broadens posteriorly and in some specimens appears darker than the rest of the medial area. Lateral carinae steep in front and pigmented across the lateral field; the carinae extend back almost to border the scutal margin (Fig. 3) and are pigmented along the ridge. Scapulae short and pointed; emargination slight.

Coxae (Figs. 4–7). Coxae flattened or only slightly convex. True coxae and not syncoxae. Coxa I prolonged by a short, broad internal spur, rounded at its tip; internal posterior margin straight and heavily pigmented. Coxa II and III unarmed, coxa IV with a very small external tuberosity. Tarsus I (Fig. 8) tapering apically with a slight hump beyond Haller's organ; tarsus IV (Fig. 9) tapering obliquely. Length of tarsus I, 0·56 mm.; metatarsus I, 0·31 mm. Length of tarsus IV, 0·50 mm.; metatarsus IV, 0·35 mm. Spiracle oval, elongate axis transverse, about same size as coxa IV; macula anteroventrally.

Genital aperture (Fig. 10). Between coxa IV (unfed specimens) or between intercoxal space of III and IV (fed specimens); genital groove diverging and extending to posterior margin.

Anus placed well posteriorly; anal groove encircling the anus, converging behind and terminating in an elongated point, and in this agreeing with *Ixodes ugandanus* Neumann 1906, *I. holocyclus* Neumann 1899 and *I. australiensis* Neumann 1904.

The females of the aforementioned species are readily distinguished by reference to the following key:

1.	Scutum distinctly broader than long, hexagonal, palps, short with article I much enlarged.	**I. australiensis**
	Scutum as long as broad, or longer than broad, not hexagonal, palps long, normal.	2
2.	Coxa I without internal spur.	**I. holocyclus**
	Coxa I with internal spur.	3
3.	No auriculae.	**I. ugandanus**
	Distinct auriculae.	**I. hoogstraali**

This work was carried out when the writer was in receipt of a Leverhulme Research Award and was a collaborative project between the author and the United States Naval Medical Research Unit no. 3, stationed at Cairo, Egypt.

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NEW SPECIES OF TICKS (*IXODES*) ASSOCIATED WITH SMALL MAMMALS

By DON R. ARTHUR

Zoology Department, King's College, University of London

(With 31 Figures in the Text)

In the course of examining some material from small mammals collected in Denmark, the British Isles and the Channel Islands, it has become evident that comparatively little is known of the species of *Ixodes* involved. A recent paper by Visotzkaya (1951) on the biology of the mouse tick, *I. trianguliceps* Birula, 1895, is the only one of its kind. Unfortunately, this paper is written in Russian and is comparatively inaccessible.

The present paper contains descriptions of new species from Lundy Island and Guernsey Island; a redescription of *I. arvicolae* Warburton, together with a description of a new variety of this species from Denmark.

Ixodes thompsoni n.sp. (Figs. 1–9)

Material examined

Holotype female and paratypes (two females) from cat, Lundy Island, 24 October 1953 (P. Davis) (in company with a female of *I. reduvius* (L)) in the private collection of G. B. Thompson at Cambridge.

Description of female

Colour of capitulum, scutum and legs in alcohol-preserved specimens very dark brown.

Body. Oval. Length (partially fed) 1·4 mm., excluding capitulum. Width 1·0 mm. Scutum does not attain halfway along body in partially fed specimens. Alloscutum abundantly supplied with long, curved white hairs, shorter white hairs on scutum.

Capitulum (Fig. 1). Length 0·71 mm., width of basis across cornua 0·37 mm. Postero-dorsal margin concave. Cornua prominent, rounded, posteriorly projecting protuberances. Lateral margins convex: corrugated as in Fig. 1. Porose areas with distinct boundaries oval in outline, separated from each other by a distance about equal to their greatest length. Palpi long but not unduly narrow, rounded apically. Outer profile lines of article II slightly concave. Inner profile lines convex, this being more marked along the distal end of article II; longer hairs confined to base of article II. Articles II:III as 1·4:1·0. Combined length of articles II and III, 0·51 mm. Palpal article I short (Figs. 2 and 3).

Ventral view. Posterior border straight, lateral borders divergent anteriorly with very slight waist behind auriculae. Latter are prominent, heavily pigmented and drawn out into downwardly and slightly backwardly pointed projections (Figs. 2–4) and in one of the paratypes are hook-like. Palpal article I drawn out into a heavily pigmented, downwardly pointed projection (Figs. 2, 3).

Figs. 1–9. Female of *Ixodes thompsoni* n.sp. Fig. 1. Dorsal view of capitulum. Fig. 2. Side view of basis to show (*a*) the auriculae, (*b*) the palpal spine. Fig. 3. Basis of one of the paratypes viewed from the front after removal of hypostome and chelicerae to show palpal projection and auriculae. Fig. 4. Ventral view of basis. Auriculae in black, ends of cornua visible from below stippled. Fig. 5. Hypostome. Fig. 6. Scutum. Fig. 7. Coxae I–IV, reading from left to right. Fig. 8. Tarsus I. Fig. 9. Tarsus IV.

Hypostome (Fig. 5). Long, narrow, slightly curved profile; ending in a rounded tip. Denticles strong laterally, arranged from base to apex as 3 rows 2/2, 7–8 rows 3/3. Corona very small, bearing small but distinct teeth. Length about 0·43 mm.

Scutum (Fig. 6). Length 1·08 mm., width 0·90 mm. Widest in front of the middle, 'lobed' marginally and terminating in a broadly rounded posterior extremity. Cervical grooves as deep, short, rapidly diverging grooves which do not extend to the postero-lateral margins. Lateral carinae present, heavily pigmented along the ridge, but this does not extend to the margin. Emargination deep, scapulae long and pointed. Punctations large and numerous.

Legs. Long, rather stout. Tarsus I (Fig. 8) short, prominently humped; tarsus IV (Fig. 9) tapering to the tip rather abruptly. Length of tarsus I, 0·50 mm.; metatarsus I, 0·28 mm. Length of tarsus IV, 0·42 mm., metatarsus IV, 0·20 mm.

Coxae (Fig. 7). Internal spur on coxa I long, stout and pointed, reaching to the anterior margin of coxa II in partially gorged specimens, appears as projected flattened corner, dark brown in colour. Internal spur of coxa I considerably longer than the external spur. Internal spur on coxa II evident as a marginal salience. External spurs present on coxae I–IV, progressively smaller from I to II, those on coxae III and IV appreciably reduced, that on IV, a marginal salience. Trochanter spurs present.

Spiracular plate. Small oval, long axis horizontal in one specimen, in two remaining specimens main axis of spiracle transverse. Macula eccentric in one specimen, central in two specimens.

Genital aperture between coxae IV.

Anus. Anal grooves do not appear to extend to posterior margin.

Male, Nymph and larva unknown

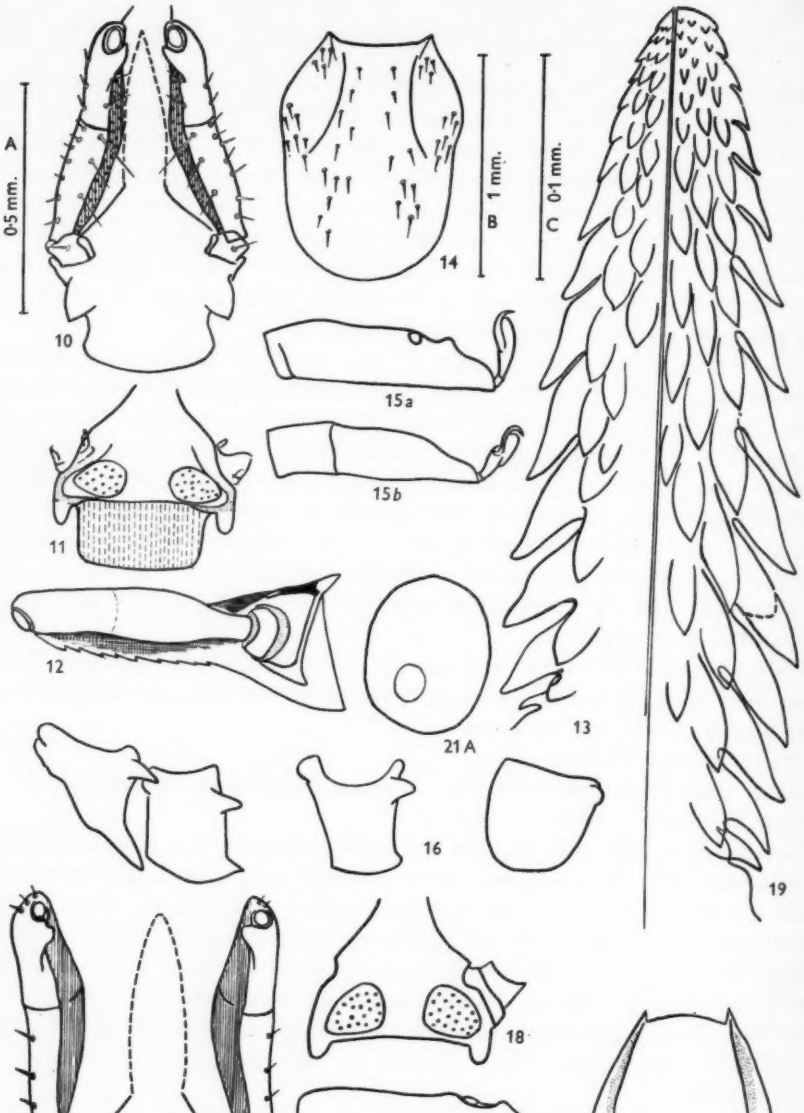
Recorded host. Domestic cat. The true host of this species is probably a bird or a small mammal. Its occurrence on the cat may be accidental. This species runs down to *I. brunneus* Koch, 1844, in Senevet (1937); to *I. frontalis* (Panzer), 1813, in Schulze & Schlottke (1929) and Schulze (in Brohmer, 1944) and to *I. neotomae* Cooley, 1944, in Cooley & Kohls (1945). *I. thompsoni* n.sp. may be separated from the *frontalis-brunneus* group by the form of the hypostome, the auriculae (cf. *I. brunneus* Cooley & Kohls, 1945), tarsi I and IV and the ventral spine of article I of the palp (see Arthur, 1953). It is a smaller species than *I. frontalis* and *I. theileri* Arthur.

Ixodes arvicolae Warburton 1926 (Figs. 17–21A, 30, 31)

A comparison between the type material of this species and the description and figures of Warburton (1926) has shown certain discrepancies. The species is therefore redescribed and figured in order to bring it into line with the other species.

Material examined

'Type' female; 3 'co-type' females, all partially engorged, England, Cambridgeshire, Quy, May 1925 (G. E. Pickford and G. E. Hutchinson).



Figs. 10–16. Female of *Ixodes guernseyensis*. Fig. 10. Capitulum, ventral view. Fig. 11. Basis capituli, dorsal view. Fig. 12. Capitulum, lateral view. Fig. 13. Left half of hypostome. Fig. 14. Scutum. Fig. 15. (*a*) Tarsus I, (*b*) Tarsus IV. Fig. 16. Coxae I–IV, reading from left to right. Figs. 17–21A. Female of *Ixodes arvicolae*. Fig. 17. Capitulum, ventral view. Fig. 18. Basis capituli, dorsal view. Fig. 19. Right half of hypostome. Fig. 20. Scutum. Fig. 21. (*a*) Tarsus I, (*b*) Tarsus IV. Fig. 21A. Spiracle.

Figs. 10, 11, 12, 15*a*, 15*b*, 16, 17, 18, 21*a*, 21*b*, 21A, to scale A. Figs. 14, 20, scale B. Figs. 13, 19, scale C.

Redescription of female

Body (partially gorged) (Fig. 31, type). Much longer than broad, elongate oval, scutum back to about one-third of body length in type and in one co-type. In remaining co-types extends back to about one-quarter of body length. Colour of capitulum and scutum pale brown, legs and palps darker in alcohol-preserved specimens; post-scutal and ventral regions with very short white hairs, not conspicuous.

Capitulum. Length from tips of cornua to apex of hypostome 0·73 mm., breadth across cornua 0·39 mm., reticulate patterning dorsally. Cornua as backwardly directed extensions (Fig. 18), bluntly rounded apically (not narrow, as illustrated by Warburton, 1926, fig. 2). Porose areas prominent, superficial, subtriangular in form and extending from the inner margin of the cornua almost to the palpal base (not 'ill-defined roughened patches', see Warburton); separated by a distance less than greatest length of areas. Palps (Fig. 17) long, sharply angled at the tip, hairs rather sparse. Length of article II:III as 2·0:1·5; combined lengths of articles II and III 0·58 mm. Outer profile lines weakly concave or straight in type and co-types, mesal border on the upper surface moderately convex. Greatest width at apex of article II. Both articles II and III hollowed out mesally, and the ventral margin below article IV is produced into a mesal knob-like projection. Ventrally basis broad, posterior edge straight; lateral margins gently convex, becoming slightly waisted behind auriculae (Fig. 17) (compare with diverging margins figured by Warburton).

Hypostome (Fig. 19). Length from basal tooth to tip of co-type specimens measures 0·39–0·40 mm. Narrow, tapering, rounded tip. Denticles arranged from proximal to distal end in about 15 rows arranged as 2 rows of 2/2, 2 rows of 3/3 and the remainder (about two-thirds of the hypostomal length) to the tip as 4/4 files, except for a small corona.

Scutum (Fig. 20). Length 1·28 mm., maximum breadth 0·83 mm. (co-type); roughly oval. Cervical grooves very faint, lateral carinae straight or gently curved, slightly divergent but not reaching postero-lateral margin. Lateral area more heavily pigmented than median and posterior areas. Median and posterior areas markedly convex. Finely punctate. Scapulae long and sharp, with mesal margins straight, not deeply emarginate. Sparse white hairs in types and co-types.

Genital aperture between third intercoxal spaces or coxae IV (dependent on degree of engorgement); anus posteriorly placed with short anal grooves, rounded in front in three co-types (not 'ogival' as given by Warburton, 1926), slightly divergent behind.

Spiracle (Fig. 21A). Oval in one of the co-types, long axis placed along longitudinal axis of body, with macula antero-ventrally. Warburton describes the spiracle as 'comparatively very large (0·35 mm.), much larger than coxa IV, broad oval, the stigma (macula) somewhat central'. This may well be so, for in other species (unpublished) there is considerable variability in both size and shape of the spiracular plate. In one of the co-types examined the spiracle was only slightly larger than coxa IV (cf. Figs. 21A and 30). Length 0·37 mm., breadth 0·30 mm.

Coxae (Fig. 30). Large, coxae I and II close together, the others further apart. Processus coxalis and processus cymati well developed on coxa I. Internal and external spurs on coxa I sharp, moderately long and approximately equal; hind-

margin of coxae I and II flattened. External spurs on coxae II and III of approximately equal dimensions, that on the rather broader coxa IV reduced to a rounded projection.

Tarsi (Fig. 21*a*, *b*). Tarsus I tapers gradually to its tip, and beyond Haller's organ there is a slight hump before the tip; length 0·59 mm. Tarsus IV moderately long, tapering rather abruptly at the end; length 0·51 mm.

Male, Nymph and Larva unknown.

Recorded host. Arvicola a. amphibius (L.) (water vole).

The 'type' and 'co-types' are in the British Museum (Natural History) collection.

Ixodes arvicolae var. **danicae** n.var. (Figs. 22–29)

Material examined

One female, unfed, from Rudehegn (North Zealand) in a mouse's nest.

Description of female

Unfed female measures 0·69 mm. at the level of the first pair of coxae, and 1·24 mm. at the level of the spiracle. Length, excluding the capitulum, is 2·26 mm. Scutum extends back for more than half body length (excluding capitulum). Body folds are wide: the dorsal rim of the spiracular plates extends to the upper surface. Short hairs present in post-scutal area and on the body folds.

Capitulum, dorsal surface (Fig. 23). Basis long, length from cornua to tip of hypostome 0·79 mm., with broad, short, pointed cornua directed backwards. Lateral margins divergent. Porose areas large and sub-triangular, separated by a distance less than their greatest width, superficial. Dorsal surface smooth, shining and impunctate. Palps long, with a slightly concave lateral profile, a smaller secondary concavity at the base of article II: strongly convex on inner surface, widest near the distal end of article II. Article II is longer than article III, as 1·4:1·0. Combined length of articles II and III, 0·6 mm., width 0·1 mm. Hairs very few, short on article I, longer and mainly on mesal dorsal surface of article II.

Ventral surface (Fig. 22). Lateral margins very gently curved, ventral surface slightly longer than broad. Auriculae large, flat, postero-laterally directed plates, broadly curved apically.

Hypostome (Fig. 24). Length 0·4 mm., uniformly tapering, gently rounded at its tip. Denticles long, narrow, tapering, progressively smaller towards the mid-line. About 14 teeth distinctly determinable in the lateral files, but small and indistinct towards the tip; dentition 9–11 rows of 4/4 teeth, extending about halfway along the hypostome, 2 rows of 3/3 teeth and 2 rows of 2/2 teeth; a bare triangular median area basally.

Scutum (Fig. 25). Length 1·27 mm., breadth 0·9 mm. Oval, cervical grooves shallow; lateral grooves rectilinear, only slightly divergent, cutting off a slightly more heavily pigmented lateral area. Surface smooth, shining, finely punctate; emargination shallow; scapulae very short, broad. Broadly rounded postero-laterally. Few fine short hairs.

Spiracular plate (Fig. 29). Particularly large, exceeding the size of coxa IV, oval

Figs. 22–29. *Ixodes arvicolae*, var. *danicae*. Fig. 22. Capitulum, ventral view. Fig. 23. Basis capituli in the region of the dorsal ridge. Fig. 24. Right half of hypostome. Fig. 25.Dorsal view of opisthosoma. Fig. 26. Ventral view of posterior half of opisthosoma to show genital and anal grooves. Fig. 27. Tarsus I and Tarsus IV. Fig. 28. Coxae I–IV, reading from left to right. Fig. 29. Spiracular plate. Figs. 30, 31. *Ixodes arvicolae*. Fig. 30. *I. arvicolae* female: coxae I–IV reading from left to right. Coxa II in this specimen possesses a slight internal spur, and there is a postero-internal marginal salience on coxa III. Fig. 31. *I. arvicolae* female—outline of Warburton's type specimen to show form of partially gorged female. (Ventral view.)

with macula situated more or less centrally, transverse axis inclined at an angle to the longitudinal body axis. Goblets very numerous. Length 0·4 mm.

Coxae (Fig. 28). Broad and flat, large. Coxa I with two short, unequal spurs; internal spur longer; separated by a straight, trenchant, dark-coloured border; processus coxalis and processus cymati well developed. Coxae II–IV trenchant with external spurs, diminishing in size from II to IV. No trochanter spurs.

Tarsi, as for *I. arvicolae*.

Genital aperture between coxae IV.

Anus very posterior, with short anal grooves, rounded in front and only slightly divergent behind.

Male, nymph and larva unknown.

Recorded host. 'Nest of mouse'.

The type will be deposited in the Danish Museum. It is with some diffidence, due to lack of knowledge of intermediate variation and material, that I have given varietal rank to this unique specimen. However, the unequal coxal spurs on coxa I, the shortness of the scapulae, the slight emargination of the scutum and the more broadly rounded posterior margin with its less convex sides, the larger spiracle and the more numerous goblets as compared with *I. arvicolae* Warburton all seem to justify such an action.

Ixodes guernseyensis n.sp. (Figs. 10–16)

Material examined

Holotype female and paratype (one female). Channel Islands (Guernsey). Deposited in the British Museum (Natural History) collection.

Description of female

Partially gorged, measures 4 mm. Oval, much broader than *I. arvicolae*.

Capitulum (Figs. 10, 11). Length 0·68 mm., width of basis across cornua 0·4 mm. Basis capituli, dorsal surface mildly convex, smooth, shining, impunctate and lacking hairs. Posterior margin straight. Porose areas roughly egg-shaped, small, extending back to dorsal ridge and forward almost to base of palp, well defined; separated from each other by a distance slightly less than their maximum length. Cornua distinct, directed backwards and upwards (Fig. 12), more or less parallel-sided seen from upper surface, rounded apically, paler in pigmentation when compared with the thickened dorsal ridge immediately in front of them. Palps sharply angled mesally at the tip. Length of article II to article III as 1·3:1·0. Combined lengths of articles II and III about 0·52 mm. Outer profile lines mildly concave; inner margin of upper surface mildly convex, inner margin of lower surface less so, with consequent asymmetrical hollowing of articles II and III. Hairs usually short along outer margin, but much longer on mid-ventral surface of article II in particular. Ventrally basis broad, rounded posterior extremity, slightly waisted peripherally and overlapped by broad, flat auriculae whose tips are directed postero-laterally.

Hypostome (Fig. 13). Length from basal tooth to tip, 0·31 mm. Narrow and tapers to point. Denticles arranged from distal to proximal end in 14 rows,

arranged as 10 rows of 3/3 and 4 rows of 2/2. Proximal third of hypostome unarmed in mid-line.

Scutum (Fig. 14). Scapulae short and pointed, emargination slight. Length 1·13 mm., breadth 0·85 mm. Suboval, widest in front of the middle, but sides more or less parallel, broadly rounded behind; cervical grooves shallow, not reaching back to postero-lateral margins. Lateral carinae present. Surface finely punctate. Hairs long and conspicuous, and similar to those on the post-scutal and ventral areas.

Legs (Fig. 15*a*, *b*). Moderately long and large. Hairs numerous and conspicuous. Tarsi long. Length of tarsus I, 0·5 mm., tarsus IV, 0·48 mm. Tarsus I humped, tarsus IV tapering.

Coxae (Fig. 16). All coxae mildly convex. Hairs few and long. Coxa I with long tapering internal spur, external spur slightly shorter but nevertheless stout. External spurs on coxae II and III shorter than that on coxa I, but larger than that of coxa IV. The latter reduced to a rounded salience. Internal spur on coxa II sharp and short, that on coxa III present as rounded thickening of coxal margin; absent on coxa IV. No trochanter spurs.

Spiracular plate. More or less rounded, diameter 0·19 mm.; macula antero-ventrally: goblets large on periphery, becoming smaller towards the macula. Four rows of goblets above macula, two rows below macula.

Genital aperture between coxae III, anal grooves straight and diverging.

Anal groove rounded in front, very slight divergent behind.

Male, nymph and larva not known.

Recorded host: 'Evotomys glareolus'.

The two specimens upon which this description is based had been determined as *I. arvicolae*.

I. acuminatus Neumann, *I. arvicolae*, *I. guernseyensis* and *I. arvicolae* var. *danicae* have so many characters in common that they may be considered as closely related. The general facies, especially the palpal characters and the basis elements, is that of a closely related group, but the differentiating characters within the group are constant in the limited material examined.

On the basis of our present knowledge, the three species and the one variety described in this paper together with *I. acuminatus* (not seen), which is only known from a few specimens collected on the Continent, may be separated by the following key (females only):

1. Laterally projecting plate arising from the basis and beneath the first palpal article. **I. trianguliceps** Birula
   No such plate. 2
2. Hypostome with a maximum of three files of denticles on the distal third at least. 3
   Hypostome with four files of denticles on the distal third at least. 4
3. Distal and ventral margin of palpal article I drawn out into a downwardly projected spine; **I. thompsoni** n.sp.
   Palpal article I without such a spine; **I. guernseyensis** n.sp.
4. Internal spur of coxa I appreciably longer than external spur. 5
   Internal and external spurs of coxa I moderately long but of about equal length. **I. arvicolae** Warburton
5. Porose areas circular, small, widely separated. **I. acuminatus** Neumann
   Porose areas oval, large, not widely separated. **I. arvicolae** var. **danicae** n.var.

This work was carried out during the tenure of a Leverhulme Research Award. Examination of the Danish material was made possible as a result of a travelling grant from the Royal Society and through the courtesy of Dr S. L. Tuxen of the Danish Museum. I am also indebted to Dr G. Owen Evans and Mr E. Browning of the British Museum (Natural History) for permission to examine the collections there and for profitable discussion, and to Mr G. B. Thompson for reading and commenting on the manuscript prior to publication.

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OBSERVATIONS ON THE COURSE OF *EPERYTHROZOON COCCOIDES* INFECTIONS IN MICE, AND THE SENSITIVITY OF THE PARASITE TO EXTERNAL AGENTS

BY JUNE P. THURSTON*

Molteno Institute, University of Cambridge

(With 5 figures in the Text)

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I. INTRODUCTION

The course of infection of *Eperythrozoon coccoides* in mice both before and after splenectomy has been described several times since the parasite was discovered by Schilling in 1928. Frequently, however, observations were made on very few mice, and some discrepancies have been found between the different descriptions. For example, Weinman (1935) detected only one parasite per 1000 red blood cells in non-splenectomized mice after inoculation, while Findlay, Klieneberger, MacCallum & Mackenzie (1939) found that young non-splenectomized mice could be infected although adults could not, and Niven, Gledhill, Dick & Andrewes (1952) concluded that eperythrozoa 'can be passed without splenectomy in normal mice more readily than is usually believed'.

This paper describes the course of infection of two strains of *E. coccoides* that were isolated in England. Some factors bearing upon the transmission of the infection and upon the resistance of the parasites to external factors are also discussed.

II. HISTORY OF THE STRAINS USED IN THE PRESENT INVESTIGATION

The first strain (Mill Hill) was isolated from the 'Parkes strain' of albino mice at the National Institute for Medical Research, Mill Hill, most of which were infected. The second strain (Molteno) was isolated from the black and brown mice bred at the Molteno Institute, Cambridge, most of which were also infected. A third strain of mice that were free of eperythrozoa was used in several experiments: this

* Tucker-Price Research Fellow of Girton College, Cambridge.

was the VS strain from Mill Hill that was mentioned by Niven *et al.* (1952) as being uninfected with *E. coccoides*.

Both strains of *E. coccoides* were similar in morphology to other strains of eperythrozoa and the organisms were very pleomorphic. When thin blood films were stained for 1 hr. with 10% Giemsa's solution the eperythrozoa appeared as coccoid bodies, as rings with frequently one or more darkly-staining granules, and as fine, curved rods with a terminal granule. The Molteno strain produced slightly larger, thicker ring forms than the Mill Hill strain. During the early stages of infection the organisms were mostly free in the plasma. They then became attached to the surface of the erythrocytes, especially of the reticulocytes, and after the height of infection had been reached the number of organisms that were free in the plasma decreased rapidly. The parasites were counted from stained blood films and the degree of infection was recorded as the number of eperythrozoa per 100 red blood cells.

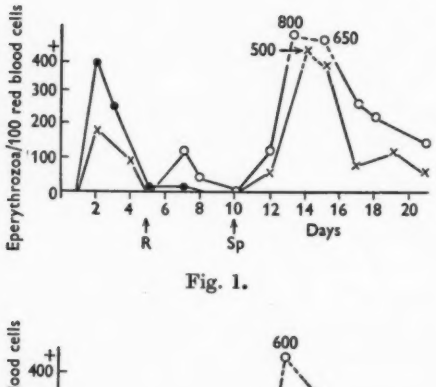
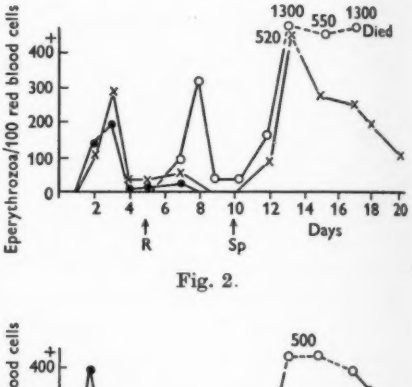


Fig. 1.



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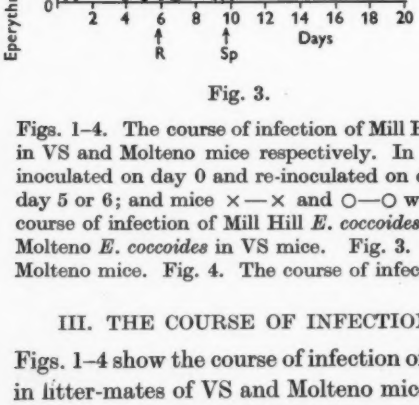

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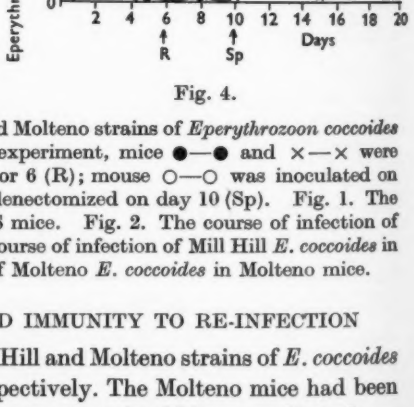

Fig. 4.

Figs. 1–4. The course of infection of Mill Hill and Molteno strains of *Eperythrozoon coccoides* in VS and Molteno mice respectively. In each experiment, mice ●—● and ×—× were inoculated on day 0 and re-inoculated on day 5 or 6 (R); mouse ○—○ was inoculated on day 5 or 6; and mice ×—× and ○—○ were splenectomized on day 10 (Sp). Fig. 1. The course of infection of Mill Hill *E. coccoides* in VS mice. Fig. 2. The course of infection of Molteno *E. coccoides* in VS mice. Fig. 3. The course of infection of Mill Hill *E. coccoides* in Molteno mice. Fig. 4. The course of infection of Molteno *E. coccoides* in Molteno mice.

III. THE COURSE OF INFECTION AND IMMUNITY TO RE-INFECTION

Figs. 1–4 show the course of infection of Mill Hill and Molteno strains of *E. coccoides* in litter-mates of VS and Molteno mice, respectively. The Molteno mice had been treated previously with doses of neoarsphenamine that should have eradicated any

latent infection. Two mice in each series were inoculated with infected blood when they weighed 11–13 g. Eperythrozoa were most numerous in the peripheral blood on the second to third day after inoculation. They then decreased in number very rapidly, and had almost disappeared by the fourth day. The height of infection was lower in the Molteno mice (Figs. 3 and 4) than in the VS mice (Figs. 1 and 2), being 40–60 eperythrozoa per 100 red blood cells compared with 180–400. One Molteno mouse (Fig. 4) had a heavy infection with 400 parasites per 100 red blood cells. It is possible that this mouse was originally uninfected while its litter-mates had been infected earlier and had retained some degree of immunity.

The mice were re-inoculated on the fifth or sixth day after the first inoculation, and at the same time a third mouse in each series was inoculated. The course of infection in the latter mice resembled the primary infection described above, but re-inoculation of the first mice caused little or no increase in the number of eperythrozoa, because of the immunity that had developed during the primary infection.

On the tenth day after the first inoculation some of the mice were splenectomized and became heavily infected. Eperythrozoa were more numerous than after the primary inoculation and persisted for longer in the peripheral blood.

These observations on the course of infection, development of immunity and effect of splenectomy are essentially the same as those previously described (for references see Weinman, 1944), except that eperythrozoa were more numerous prior to splenectomy. The strains of *E. coccoides* isolated at Mill Hill and at the Molteno Institute appear to be identical in behaviour, and mice that recovered from infection with one strain were immune to cross-infection with the other. The height of the infection prior to splenectomy is apparently dependent more on the susceptibility of the strain of mice than on the virulence of the strain of eperythrozoa.

Two mice were still immune to re-infection $3\frac{1}{2}$ months after inoculation. This agrees with the finding of Weinman (1935) that immunity lasts for several months. Immunity probably remains throughout the chronic stage of infection, when eperythrozoa can be detected in the blood only occasionally.

IV. THE RATE OF MULTIPLICATION OF *EPERYTHROZOON COCCOIDES*

During the experiments on the course of *Eperythrozoon* infection in mice, it was noticed that the number of parasites fluctuated considerably. For example, as shown in Fig. 1, eperythrozoa were found only rarely in one mouse on the day after inoculation, but there were 400 parasites per 100 red blood cells at the next blood examination on the second day. As it was considered that this large number might have arisen either by simple multiplication of the parasites already in the blood or by the sudden liberation into the blood of parasites that had multiplied elsewhere, two further mice were examined at frequent intervals for 60 hr. to find whether the number of parasites did in fact increase continuously or discontinuously.

The course of infection in these two mice is shown in Fig. 5. The mice had been inoculated $3\frac{1}{2}$ days earlier, and by the beginning of the experiment the number of parasites had decreased after the primary infection. The number of eperythrozoa in the blood of each mouse remained fairly constant during the first four examinations.

The mice were then splenectomized and the parasites subsequently increased in number. The increase was slow for the first 8 hr. but then became very rapid, although apparently quite continuous. The most rapid increase was found in mouse A, in which the number of eperythrozoa increased from 188 at 35 hr. 30 min. to 643 at 44 hr. 10 min. At this rate, the number of eperythrozoa was doubled in about 5 hr., a rate which is similar to that of many bacteria and which is quite compatible with continuous multiplication. When the peak of infection had been reached the number of parasites decreased rapidly.

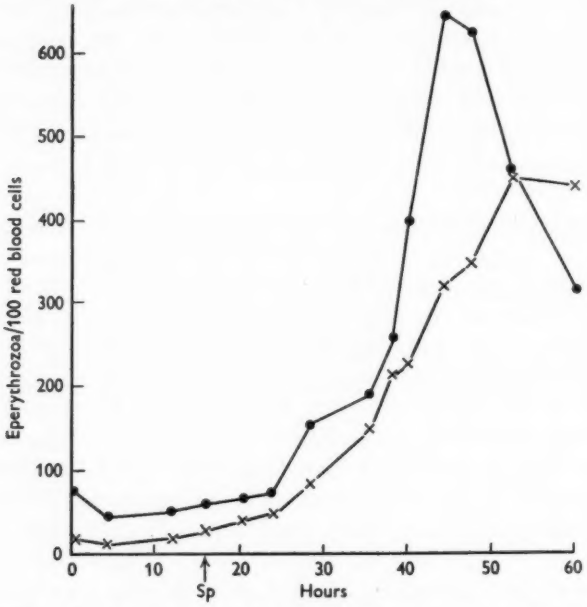

Fig. 5. To show the rapid increase in number of *Eperythozoon coccoides* after splenectomy (Sp).

V. ATTEMPTS TO TRANSMIT IMMUNITY

In an attempt to transmit an immune factor from infected mice, serum was taken from mice when the number of eperythrozoa began to decrease. It was injected twice daily into newly infected VS mice but had no effect on the course of infection. Similarly, Bruynoghe & Vassiliadis (1929*a*) found no protective substances in serum from infected rabbits when tested in mice, and Eliot & Ford (1930) found none in serum from infected rats when tested in other rats.

Dinger (1929) and Bruynoghe & Vassiliadis (1929*b*) found that the injection of a spleen suspension had no effect on the course of *E. coccoides* infection. In the present experiments, three mice were splenectomized, and the excised spleen of each mouse was immediately transplanted in the same mouse, either subcutaneously or between the peritoneum and the body wall. The presence of the spleen tissue in this position similarly had no effect on the course of infection, which became as acute as after a normal splenectomy. The transplanted tissue appeared healthy at autopsy 7 days later.

Weinman (1935) concluded that no immunity was transmitted from parent to offspring because young mice of carrier stock were susceptible to infection. In order to check this, an experiment was performed with two litters of eight baby mice. The mother of one litter was of uninfected VS stock. The other mother had initially a low, chronic infection of *E. coccoides* but was splenectomized on the day after the birth and a heavy blood infection developed and persisted for several weeks. When they were 5 days old, four of the litter from the infected mother were exchanged for four baby mice (3 days old) from the VS mother, so that each mother suckled 4 baby mice from her own litter and 4 from the other litter.

The baby mice were examined 3 days after the exchange and were all uninfected. Blood films also appeared negative when the mice were 3 weeks old, at which time they were weaned and 2 were put into each of 8 cages, thus—cages 1 and 2 contained mice born to the VS mother and suckled by the infected mother; cages 3 and 4 contained mice born to the VS mother and suckled by her; cages 5 and 6 contained mice born to the infected mother and suckled by the VS mother; and cages 7 and 8 contained mice born to the infected mother and suckled by her.

The mice in cages 1, 3, 5 and 7 were inoculated with *E. coccoides*. Acute infections developed in 2 days in all the mice, thus showing that no immune factor had been transmitted through the milk. The mice in cages 2, 4, 6 and 8 were splenectomized and were all found to be uninfected, thus showing that no infection had been transmitted through the milk, and that in this experiment there had been no congenital transmission of infection.

In other experiments, baby mice were inoculated with *E. coccoides* and developed acute infections while they were being suckled by their infected mothers.

VI. ATTEMPTS TO TRANSMIT INFECTION

It has been shown above that infection is not transmitted through milk. This was also found by Weinman (1935) who, as he could not infect three mice by feeding them with blood or infected organs, believed that the failure of transmission through milk might be attributed to the difficulty of infecting by the gastro-intestinal route. In the present experiments, however, five out of eight young or splenectomized VS mice were infected by citrated blood given by mouth. Therefore, the absence of parasites from milk is the most likely reason for the lack of infection in suckling mice.

Citrated blood did not infect when it was smeared on the external mucous membranes—eyes, nostrils and urino-genital surfaces, although very low concentrations of infected blood were sufficient to infect susceptible mice when injected intraperitoneally or intravenously. There was little difference in the speed, height or constancy of infection resulting from these two routes. In three out of five experiments, as little as 0·2 ml. of a 1:100,000 dilution of infected blood in citrate-saline infected VS mice.

Urine and a suspension of faeces from infected mice did not infect VS mice when injected intraperitoneally. Splitter (1952) similarly found that urine from pigs infected with *E. suis* was non-infective.

VII. THE EFFECT OF TEMPERATURE, DRYING, LYSIS AND CHEMICAL AGENTS ON THE INFECTIVITY OF *EPERYTHROZOON COCCOIDES*

This study was carried out to ascertain the resistance of eperythrozoa to external factors, as such knowledge is necessary when investigating possible means of transmission, or when handling infected material *in vitro*, or when sterilizing instruments that have become contaminated.

Preparations to be tested were injected intraperitoneally into VS mice, which were examined 2 and 4 days later. If no eperythrozoa were then found in blood films, the mice were splenectomized and were examined 2 and 4 days after splenectomy. If blood films were still negative, the mice were then inoculated with another experimental preparation and examined for a further 4 days. If they remained uninfected, the mice were then inoculated with infected blood, and in each case infections became patent 2–3 days after inoculation, showing that the mice were susceptible to infection.

The effect of temperature. Citrated blood remained infective after being kept at 3° C. for 11 days, but was not infective after 14 days. Weinman (1935) similarly found that citrated blood was infective after 5 days at 5° C., but was not infective after 10 days; and Splitter (1952) showed that defibrinated blood containing *E. suis* was infective after 31 days at −32° C.

Citrated blood was still infective after being kept at room temperature (16–17·7° C.) for 17 hr., or at 37° C. for 3 hr. It was not infective after 24 hr. at 16–17·7° C. or 24° C., or after 5 hr. at 37° C. The adverse effect of the higher temperature is possibly the result of the eperythrozoa consuming some essential metabolite from the blood more quickly than at lower temperatures, and therefore being unable to survive as long as at the lower temperature. However, glucose or glutamine (each 2·0 mg. in 1 ml. citrated blood) did not prolong the infectivity of the eperythrozoa at 37° C.

The effect of drying. A drop of infected blood on a glass slide was left to dry for 24 hr. at 3° C. It was then mixed with citrate saline and injected intraperitoneally into VS mice, but did not infect them. Eperythrozoon infections are therefore unlikely to be transmitted through contact with dried infected blood.

The effect of lysis. Infected blood was mixed with twice its volume of distilled water, so that most of the red blood cells were lysed. It was left at room temperature for 30 min. and was then stored at 3° C. The eperythrozoa were as infective as normal after 1½ hr. and after 24 hr., showing that they were able to survive in a very hypotonic solution.

The effect of chemical agents. The effect of phenol on *E. suis* was investigated by Splitter (1950). He added 0·5% phenol to defibrinated infected blood and stored it at 37° F. for 15 days, as for hog cholera vaccine, to find whether this was a possible source of transmission of the infection. The blood was not infective when tested in susceptible swine.

This experiment was repeated with *E. coccoides* by adding 0·5% phenol to infected citrated blood and storing it at 3° C. The preparation was injected into VS mice after 1 hr., but no infection resulted, indicating that the phenol had rapidly destroyed the eperythrozoa.

It has previously been shown (Thurston, 1953) that neoarsphenamine, 0·3 mg. per 20 g. mouse, intraperitoneally, caused eperythrozoa to disappear from the blood of mice within 2–3 hr. after its injection. The action of the drug was inhibited if the mice were treated with dimercaprol, BAL (2·0 mg. per 20 g. mouse, subcutaneously, in arachis oil) 1½ hr. before being treated with neoarsphenamine and again 30 min. after treatment.

In order to find whether the arsenical acted directly upon the eperythrozoa, 2·0 mg. of neoarsphenamine was added to 2·0 ml. of citrated infected blood and the preparation was stored at 3° C. A further sample of the citrated infected blood was stored as a control. After 2, 5, 16 and 26 hr., 0·2 ml. of the preparation or of the control material was injected into VS mice that were treated with BAL according to the schedule described above. This treatment with BAL was carried out to prevent the neoarsphenamine from acting upon the eperythrozoa *in vivo*.

The mice which were inoculated after 2 and 5 hr. developed blood infections similar to those in the mice which were inoculated with the control material; i.e. neoarsphenamine did not appreciably affect the infectivity of the preparation in 5 hr. The mice which were inoculated after 16 hr. developed blood infections, but the infection did not become patent until 2–3 days after the height of infection was reached in the controls, i.e. neoarsphenamine had appreciably reduced the infectivity of the preparation in 16 hr. No eperythrozoa were found in the mice which were inoculated after 26 hr. They were examined for 5 days after inoculation and were then splenectomized and examined for a further 6 days. Neoarsphenamine, therefore, destroyed the infectivity of eperythrozoa in 26 hr. Although neoarsphenamine eventually destroyed the infectivity of the eperythrozoa *in vitro*, its effect was not as dramatic as its action *in vivo*, and was not as rapid as the effect of phenol.

VIII. THE EFFECT OF CORTISONE AND OF CONCURRENT INFECTIONS ON INFECTIONS OF *EPERYTHROZOON COCCOIDES*

It has been reported that cortisone increases the degree of infection of *Plasmodium cynomolgi* in monkeys and evokes relapses of latent infections of this parasite in the same way as does splenectomy (Schmidt & Squires, 1951), and that it likewise increases the degree of infection of *P. berghei* in mice (Findlay & Howard, 1952). Its action on these parasites has, however, been denied by Schneider (1953) and by Roberts (1954).

The effect of cortisone was tested upon infections of *E. coccoides* in mice, when it was given either at the time of inoculation or when the infection had become latent after the primary infection. In both series, 1·0 mg. cortisone per 20 g. mouse was given subcutaneously once daily for 6–9 days, but this dose had no effect on the course of infection. It neither lengthened the acute phase of the infection nor evoked a relapse, although the infection always relapses after splenectomy.

It has been reported that *E. coccoides* suppresses concurrent infections of *Haemobartonella* in white mice (Weinman, 1944) and that both *Haemobartonella* and *Eperythrozoon* infections are affected by concurrent infection with other micro-organisms. Latent infections of *Haemobartonella muris* were brought out by con-

current infection with trypanosomes (Mayer, Borchardt & Kikuth, 1927; Rosenthal & Zohman, 1931), and latent infections of *E. coccoides* relapsed during infections of *Spirochaeta recurrentis* (Dinger, 1929) and of lymphatic leukaemia (Marmorston, 1935). *E. coccoides* acted synergistically with the S virus to produce fatal mouse hepatitis and the eperythrozoa increased in number until the death of the host (Niven *et al.* 1952). Heavy infections of *E. coccoides* were also observed in mice with *Babesia canis* infections (Ryley, personal communication).

During the present investigations, *E. coccoides* was frequently observed during infections of *Plasmodium berghei* in mice. As the normal strain of *P. berghei* was contaminated with *E. coccoides*, experiments were planned to find whether the *Eperythrozoon* infection was affected by the *P. berghei*, or whether the effect seen was merely the normal course of infection of the *E. coccoides* that was present in the infective inoculum. The strain of *P. berghei* was freed from the *E. coccoides* for these experiments by passing the infection through mature rats for three passages, instead of through mice, because rats are less susceptible to infection with eperythrozoa.

Table 1. *The effect of* Plasmodium berghei *on infections of* Eperythrozoon coccoides

	Experiment 1				Experiment 2				Experiment 3					
	Mouse A		Mouse B		Mouse C		Mouse D		Mouse E		Mouse F		Mouse G	
Day	Ec.	Pb.	Ec.	Pb.	Ec.	Pb.	Ec.	Pb.	Ec.	Pb.	Ec.	Pb.	Ec.	Pb.
2	—	—	—	—	290	0·2	20	0·1	20	—	50	—	25	—
3	310	0	185	0	75	2	5	1	40	—	80	—	60	—
4	60	0·5	35	1	0	11	0	7	—	—	—	—	—	—
5	5	4	2	5	1	33	0	24	0	—	0	—	0	—
6	—	—	—	—	20	56	0	35	—	—	—	—	—	—
7	—	—	—	—	45	76	0	56	0	—	0	—	0	—
8	—	—	—	—	240	80	1	65	—	—	—	—	—	—
9	—	—	—	—	—	—	4	70	0	0·2	0	0·3	0	0·5
10	—	—	—	—	—	—	—	—	10	3	0	4	0	2
11	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—	15	50	30	60	30	28
13	—	—	—	—	—	—	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—	50	82	90	88	80	75

Ec., *Eperythrozoon coccoides*, number of parasites per 100 red blood cells; Pb., *Plasmodium berghei*, number of parasitized red blood cells per 100 red blood cells.

In the first two experiments (Table 1), mice were inoculated with both *E. coccoides* and *P. berghei*. As in previous experiments (Figs. 1–4), the *E. coccoides* infection reached a peak in 2–4 days and then decreased rapidly; at this time *P. berghei* parasites became apparent in stained blood films. In the first experiment, the mice (A and B) were not examined after the fifth day. In the second experiment, the mice (C and D) were examined daily until death. Eperythrozoa increased in number again in mouse C when the infection with *P. berghei* became heavy, and numerous eperythrozoa were found shortly before the death of the

mouse. The eperythrozoa were as numerous on red blood cells infected with *P. berghei* as on uninfected red blood cells. Whereas all the other mice shown in Table 1 were of VS stock, mouse D was of Molteno stock and probably had a natural latent infection and immunity. The primary infection with *E. coccoides* in this mouse was very slight, and no further eperythrozoa were detected in blood films until the *P. berghei* infection was very heavy.

In the third experiment, mice E–G were inoculated with *E. coccoides* alone. After the primary infection the blood films became negative and then, 7 days after the inoculation with *E. coccoides*, the mice were inoculated heavily with *P. berghei*. The *E. coccoides* infection relapsed when the *P. berghei* parasitaemia increased.

From these experiments it is concluded that the presence of *P. berghei* in the blood has no effect on the course of infection of *E. coccoides* until the infection of *P. berghei* is so heavy that the reticulo-endothelial system is blocked. When this occurs, it causes relapses of *E. coccoides*. Rosenthal & Zohman (1931), had previously shown that relapses of *Haemobartonella muris* were evoked when the reticulo-endothelial system of rats was blocked by indian ink.

The course of *P. berghei* infection uncontaminated with eperythrozoa is not shown in the table, but it was found that the concurrent infection with *E. coccoides* had no effect on the course of infection of *P. berghei*.

IX. DISCUSSION

It is thought that eperythrozoa are probably transmitted in nature by biting arthropods (Weinman, 1944; Splitter, 1950) and the non-infective nature of the urine, faeces and dried blood in the present studies similarly suggests that blood inoculation is the most usual means of transmission. Eliot (1936) successfully transmitted *E. coccoides* by means of the louse *Polyplax serrata*; this was probably a case of mechanical transmission because the lice became non-infective if several hours elapsed between feeding on the infected mouse and feeding on the susceptible mouse. In deciding between mechanical and cyclical transmission it should be remembered that eperythrozoa survive for at least 17 hr. at room temperature and that the vector should, therefore, be maintained for more than 24 hr. before mechanical transmission can be excluded.

Although eperythrozoa are affected by only a few drugs *in vivo* (Thurston, 1953) they are sensitive to the action of several different chemicals and to change of temperature when tested *in vitro*. It is interesting to note that eperythrozoa remained infective in a very hypotonic solution.

The interaction of parasites is a matter of great interest, especially with parasites such as *E. coccoides* which may infect whole populations in a latent condition and yet not be recognized until after splenectomy or during a concurrent infection. Relatively low, long-continued infections of *E. coccoides* are sufficient to cause anaemic changes in mice (Thurston, 1954), and as such chronic infections may be evoked by a concurrent infection it is possible that the anaemic changes caused by the eperythrozoa could be attributed to the concurrent infection without their actual cause being discovered. In the present experiments, eperythrozoa were

evoked only during the terminal stages of *Plasmodium berghei* infection and the above considerations do not apply, but they remain an interesting theoretical possibility in diseases of animals and man.

X. SUMMARY

Non-splenectomized mice developed acute infections of *Eperythrozoon coccoides* after inoculation, but were then immune to re-infection. Splenectomy evoked acute infections that were heavier and longer in duration than the primary infection; during this phase, the parasites doubled in number in about 5 hr. The presence of splenic tissue transplanted subcutaneously did not prevent this evocation by splenectomy.

The number of eperythrozoa decreased rapidly after the peak of infection had been reached, but no immune factor was detected in the blood. No immune factor or infection was transmitted from mother to offspring through the milk.

Citrated blood remained infective for 11 days at 3° C., for 17 hr. at 16–17·7°C. and for 3 hr. at 37° C. It was not infective after 24 hr. at either 24° C. or 16–17·7°C., or after 5 hr. at 37° C. Parasitized blood was not infective after drying.

Citrated blood was infective to mice when given by mouth, but was not infective when applied externally. Urine and faeces were non-infective.

Eperythrozoa remained infective when infected blood was diluted with twice its volume of water and left for 24 hr. at 3° C. Phenol, 0·5 %, rendered citrated blood non-infective in 1 hr. Neoarsphenamine, 0·1 %, reduced the infectivity of citrated blood in 16 hr., and rendered it non-infective in 26 hr.

Cortisone did not affect the course of infection of *E. coccoides* in mice when given subcutaneously, 1·0 mg. per 20 g. mouse, once daily for 6–9 days.

Concurrent infection with *Plasmodium berghei* evoked relapses of *E. coccoides* during the terminal stages of the *P. berghei* infection.

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STRAIN VARIATION IN *ENTAMOEBA HISTOLYTICA*

I. CORRELATION OF INVASIVENESS IN RATS WITH THE CLINICAL HISTORY AND TREATMENT OF THE EXPERIMENTAL INFECTIONS

By R. A. NEAL and PATRICIA VINCENT

The Wellcome Laboratories of Tropical Medicine, London

Variation in virulence between strains of *Entamoeba histolytica* has been demonstrated experimentally by several workers, but a correlation with the case history of the patient from whom the strain was obtained has not been conclusively proved; thus Anderson, Bostick & Johnstone (1953), in their recent monograph on amoebiasis, conclude (p. 46) that 'the existence of strains (independent of cyst size) of more virulent *E. histolytica* is not unequivocally established. Some excellent authorities support this thesis, whereas other eminent workers deny that more virulent strains have been objectively demonstrated.'

In a previous investigation (Neal, 1951*a*) strains of *E. histolytica* were examined in the rat for their power to invade the caecal wall, when it was found that those isolated from acute cases of human amoebiasis produced ulceration while those from symptomless carriers—with one exception—did not invade the caecal wall. As the number of strains used was insufficient to reveal any significant correlation with the human infection, a further series has been examined, with particular emphasis upon those from symptomless cases. The present paper deals with the invasiveness of these strains in the rat, and their susceptibility to treatment with emetine.

In this and subsequent papers on this subject, the term invasiveness will be used to denote the virulence of *E. histolytica*. By this is meant the power of this amoeba to invade the caecal wall and produce ulceration. Each strain will therefore be described as invasive or non-invasive, the determination being based on the average caecal score (see p. 154) in rats infected with the strain in question, those with an average score of 2·00 or less being regarded as non-invasive. It is important to realize that these terms are only relative, since even the avirulent strains occasionally produce ulceration. However, as can be seen from data published previously (Neal, 1951*a*) and from the present results, the difference between the average caecal scores of strains termed invasive and non-invasive is extremely marked. This nomenclature has also been discussed elsewhere (Neal, 1954).

MATERIAL AND METHODS

(*a*) *Strains of* Entamoeba histolytica

The strains from contact carriers were isolated from faeces containing cysts in the manner described previously (Neal, 1951*a*), while those from acute cases were isolated from diarrhoeic stools containing amoebae. They were not used for experimental infections until the amoebae were completely established *in vitro*. Experience has shown that at least ten subcultures are necessary for the balance between the amoebae and bacteria to become adjusted so that there is regular and

abundant growth of the protozoan. Each strain was generally propagated by subculturing twice a week. In most, the formation of cysts occurred fortuitously, but a few strains—those marked with an asterisk (*)—were maintained by encystment (see also Neal, 1954). The morphology of all these strains was that of typical *E. histolytica* and the cysts were found to be of the large race.

Contact carrier cases

1. *AI**. Pakistani. Strain cultivated from 23. i. 50 to 6. x. 50.
2. *ZM**. Pakistani. Strain cultivated from 23. i. 50 to 6. x. 50.
3. *Zada**. Pakistani. Strain cultivated from 2. iv. 52 to 22. ix. 54.
4. *SI*. Pakistani. Strain isolated on 8. x. 53 and still under cultivation.
5. *GH*. Pakistani. Strain cultivated from 9. vii. 53 to 9. x. 53.
6. *SH*. Pakistani. Strain cultivated from 9. vii. 53 to 8. ix. 53.
7. *EA*. British. Served in Hong Kong and was invalided home with sprue; amoebiasis found on routine examination; mucosa was normal when examined by sigmoidoscopy. Strain isolated on 23. vi. 52 and is still under cultivation; parallel line maintained by encystment, named *EAC*, also still being cultivated.
8. *GA*. British. Invalided home from Fayid, Egypt with fractured phalanx and divided extensor tendon. Routine faecal examination revealed amoebiasis, mucosa was normal on sigmoidoscopy. Strain cultivated from 1. x. 52 to 2. iii. 54.
9. *ND*. British. Resided in Kenya, amoebiasis found on routine faecal examination. Strain isolated on 24. iv. 53 and still under cultivation.
10. *HK*. British. Served in Korea; cysts found on routine faecal examination. Strain cultivated from 11. i. 54 to 15. xi. 54.
11. *HA*. British. Invalided home from abroad with psychiatric trouble. *E. histolytica* cysts were found in the faeces on routine examination. Strain cultivated from 27. i. 54 to 16. iii. 54.

Specimens from which strains nos. 1–6 were isolated were sent to us by Group-Captain W. P. Stamm, who discovered these cases amongst a group of Pakistani air-apprentices under training with the R.A.F. These air-apprentices had resided in this country for 1–3 years, during which time none of them had reported any symptoms due to amoebiasis. Cases nos. 7–11 were servicemen invalided home with various ailments, but none gave any history of symptoms referable to his amoebiasis. Case no. 8 suffered two attacks of diarrhoea during 1940 and 1952 which were treated in Kenya. When the present strain was isolated no symptoms were present and *E. histolytica* was discovered only on routine faecal examination.

Acute cases

12. *OW*, 13 *MC*. These strains may be considered together as they were isolated from two cases of acute amoebiasis among a series of such cases found in British R.A.F. personnel at a camp in England (Morton, Stamm & Seidelin, 1952). The strains were cultivated from 7. ii. 51 to 5. vi. 52 and 29. i. 51 to 13. xii. 51 respectively.

14. *RS*. Pakistani. Strain cultivated from 8. vii. 53 to 1. iii. 54; it never grew regularly and satisfactorily *in vitro*. This case is not perhaps accurately classified as suffering from acute amoebiasis, but some symptoms were found, showing that the disease was active.

(*b*) *Animals used, method of inoculation and assessment of results*

Rats were used exclusively and were inoculated by injection of *E. histolytica* amoebae into the caecum after laparotomy. The strain of rats used, method of inoculation and examination for infection were similar to those employed previously (Neal, 1951*a*, *b*). Briefly, the technique consisted of inoculating the culture of amoebae intracaecally, then 7 days later killing the animals and examining the caecum and colon for the presence of amoebae and for ulceration of the intestinal wall. The number of amoebae inoculated was not determined accurately, but a drop of the inoculum examined microscopically ($\frac{2}{3}$ in. objective, 10 × ocular) contained more than ten amoebae per field. As in the previous papers, the degree of caecal ulceration of each rat was assessed by a scoring method. A score of 0 to 4 was awarded according to the extent of the ulceration of the wall and to the caecal contents according to the proportion of mucus present. In the present work these two figures are combined thus giving a maximum possible score of 8 for each rat.

(*c*) *The presence of microscopical ulceration in the caecum*

As will be discussed later (p. 159) there is some controversy as to whether microscopic lesions are present in the absence of macroscopic ulceration. In an attempt to resolve this question, the caeca of two rats, one infected with strain *EA*, the other with *EAC*, were sectioned in the following manner.

Each caecum was split open and gently rinsed in normal saline to remove most of the contents, then fixed in Bouin's solution overnight (about 16 hr.). The tissue was then embedded in paraffin wax in the usual way and serial sections 10 μ in thickness were cut. Out of every forty, ten or more consecutive sections were stained with Erhlich's haematoxylin and eosin and mounted. The slides were examined microscopically using the $\frac{1}{6}$ in. objective and 10 × and 6 × eyepieces. Both of these animals were heavily infected with *E. histolytica*; a smear of the caecal contents taken before fixation showed more than five amoebae in each field ($\frac{1}{6}$ in. objective, 10 × ocular). Macroscopically, the caeca appeared absolutely free of ulceration and they were fixed within 5 min. of the rats' death.

RESULTS

(*a*) *Virulence*

Table 1 shows the effect of inoculating rats with strains of *E. histolytica*. It can be seen from this table that in one series of strains the amoebae were non-invasive to the rats (strains nos. 1 to 11), while the remaining three showed invasive properties (strains nos. 12 to 14), the last (no. 14) being less invasive than the other two strains. The virulence of these strains was not connected with their infectivity since all strains infected more than 70 % of the rats. It should also be noted that the non-invasive strains, with the exception of strains nos. 5, 10 and 11, all

occasionally ulcerated the caecum, and the ulceration in a few of these animals was as great as that seen with the invasive strains *OW* and *MC*.

Strain *RS* (no. 14) showed definite invasive properties (the caeca of fourteen out of twenty-one infected rats were ulcerated), but the extent of the ulceration was not great, as is shown by the average caecal score of 3·29 out of 8·00 (maximum).

The results obtained with two strains from acute cases *OW* and *MC* are included in this report for comparison with those obtained from symptomless cases. Amoebae from strains *OW* and *MC* proved infective and invasive to rats as did those from two other strains which were isolated from cases of human amoebiasis showing symptoms. Two strains of *E. histolytica* which came from symptomless cases were not infective to rats and remained non-infective during the period of cultivation.

Table 1. *The infectivity and ulceration of the caecum of rats infected with various strains of* Entamoeba histolytica

Strain of *E. histolytica*		No. of rats infected/ inoculated and percentage	No. of rats with lesions	Average caecal score of infected rats ± standard error
Name	No.*			
AI	1	16/20 (80)	2	1·19 ± 0·42
ZM	2	15/21 (71)	5	1·83 ± 1·19
Zada	3	18/19 (95)	1	0·33 ± 0·41
SI	4	14/16 (88)	1	0·79 ± 0·40
GH	5	7/7 (100)	0	0
SH	6	6/7 (86)	1	1·00 ± 0·58
EA	7	31/38 (82)	3	0·84 ± 0·37
GA	8	6/8 (75)	1	1·00 ± 1·55
ND	9	23/32 (72)	3	0·70 ± 0·38
HK	10	15/17 (88)	0	0
HA	11	4/5 (80)	0	0·75 ± 1·06
OW	12	11/11 (100)	11	7·91 ± 0·14
MC	13	23/26 (88)	23	7·87 ± 0·19
RS	14	21/24 (88)	14	3·29 ± 0·58

* Serial number of strains from page 153.

(*b*) *Treatment with emetine hydrochloride*

The treatment of non-invasive strains with emetine hydrochloride revealed some differences in susceptibility between strains. Two strains (nos. 5 and 6) were susceptible to 1 mg./kg. of emetine, whilst another (no. 2) was not completely eradicated by the highest non-toxic dose, 4 mg./kg. Another strain (no. 10) was not affected by 2 mg./kg., though it succumbed to 4 mg./kg. The remaining non-invasive strains were eradicated by 2 mg./kg.

Rats infected with invasive strains *MC* and *OW* (nos. 12 and 13) were not all completely cleared of amoebae by 4 mg./kg. of emetine. Strain *RS* (no. 14) showed some response to treatment with 2 mg./kg., but complete data were not obtained with this strain.

(*c*) *Examination of caeca of infected rats for microscopic lesions*

The caeca of two rats infected with strains *EA* and *EAC*, in which no ulceration was detected macroscopically, were sectioned in the manner described above.

Microscopical examination of these sections revealed that no lesions or ulceration were present though smears of the lumen contents revealed numerous amoebae. Microscopical examination of sections of the caecal wall showed that amoebae were often in contact with the mucous membrane and more were within a distance of 10 μ from the surface of the mucosa. However, the glands were perfectly normal in structure and even such a minute detail as the striated border of columnar epithelial cells was observed to be intact. Other evidence for the absence of tissue feeding was obtained by inspection of the food vacuoles in the amoebae, which

Table 2. *Treatment of rats infected with various strains of* Entamoeba histolytica *with emetine hydrochloride*

Strain of *E. histolytica*		Proportion of rats cleared/inoculated, by dose of emetine (mg/kg. × 6)			
Name	No.*	4	2	1	0·5
AI	1	—	20/25	15/17	—
ZM	2	3/7	6/19	5/16	—
Zada	3	—	7/7	5/12	3/12
GH	5	—	6/6	6/6	—
SH	6	—	—	6/7	—
EA	7	—	7/9	8/14	1/5
GA	8	—	8/8	6/8	—
ND	9	18/18	17/19	9/16	—
HK	10	10/10	2/10	1/10	—
OW	12	7/10	1/11	2/11	—
MC	13	11/13	5/15	1/7	—
RS	14	—	3/7	2/6	—

* Serial number of strains from p. 153.

contained only bacteria and yeasts. Sometimes minute depressions, about 5 μ in depth and width, could be seen in some apical cells which could have been caused by an amoeba, but the nucleus and remainder of the cytoplasm of the cell was quite normal. These depressions were also observed in other parts of the section in the absence of amoebae. On only three occasions was an amoeba observed lying between the glands, but even then they were not more than a quarter of the total length of the gland from the lumen.

Sometimes the tips of a series of neighbouring glands were missing but no amoebae were seen within the tissue, and the glands in the corresponding area in adjacent sections were intact. Amoebae lying in debris covering the gland on either side of the suspected lesion were feeding on bacteria and yeasts as previously mentioned. These considerations, and the general appearance, suggest that the tips of the gland had been torn away during preparation of the slide.

DISCUSSION

The results obtained in the present work show that *E. histolytica* is highly infective to young rats and the invasiveness varies with different strains. These conclusions therefore confirm previous work (Neal, 1951*a*, *b*). It was also concluded from the earlier results that the more virulent strains gave a higher infectivity rate; this

seems now to be incorrect as the present results show that the infectivity rate of non-invasive strains may reach 100%.

The Pakistani air-apprentices were undergoing training in this country for 3 years, and the only one in the series under examination to fall sick with an intestinal complaint, was the case from whom strain *RS* (no. 14) was isolated. All strains from the Pakistanis were non-invasive in the rats except strain *RS*, which showed definite invasive properties, though to a lesser degree than those isolated from acute cases (strains nos. 12 and 13). The data obtained with strain *EA* (no. 7) require some comment since this case suffered abroad from intestinal trouble which was diagnosed as sprue. On arrival in Great Britain, routine examination revealed the presence of *E. histolytica* cysts in the faeces. It is uncertain which disease caused these symptoms as specific treatment for amoebiasis was given and sprue tends to spontaneous cure in this country. However, if the symptoms were due to the parasitic infection, one would expect the culture to manifest invasive properties in the rat, but since it proved to be non-invasive, it would appear that the symptoms were unrelated to the amoebic infection.

Efforts were made to discover whether the manner of conducting the experimental infections might account for the variations of invasiveness between strains, but no technical errors could be detected. In addition, the possibility that some strains produced ulceration more slowly than others was not upheld (Neal, 1951*b*), and the mode of propagating *E. histolytica in vitro* was not found to exert any influence on the invasiveness (Neal, 1954). The conditions of experiment have been rigidly standardized.

Ishii (1951), working with experimentally infected rats, observed that amoebic ulcers were often found at the site of injection and suggested that injury to the mucosa is involved in the pathogenesis of *E. histolytica*. This is considered unlikely to be the principal factor, as in the present work very few lesions were found in rats infected with non-invasive strains, and injury to the mucosa is unavoidable with the present method of inoculation.

We now turn to the correlation of invasiveness in the rat of different strains of *E. histolytica* with the history of the case from which they were isolated.

In the present paper, three strains from acute cases proved invasive to rats, while eleven strains from symptomless cases proved non-invasive. If results published in a previous paper (Neal, 1951*a*), obtained by the use of an identical technique, are included the following figures are obtained. All six strains isolated from patients with symptoms, showed invasive properties, while thirteen strains from carriers were non-invasive in the rats. One strain from a carrier was as invasive as those from acute cases, but sigmoidoscopy of this patient revealed evidence of healed amoebic lesions and therefore throws doubt on the classification of this case as a carrier (Neal, 1951*a*). There is, therefore, a good correlation between the presence or degree of invasiveness of *E. histolytica* in the rat with the type of amoebiasis present in the human host at the time of isolation of the strain.

This conclusion is in marked contrast with the prevailing view (see Anderson *et al.* 1953) that ulceration occurs in every human infection with *E. histolytica*.

This view is based upon the result of autopsies performed on people who had died from other causes and upon experimental infections mainly using kittens as laboratory animals. The latter evidence will now be examined and is restricted to those papers where a series of strains are compared with one another.

Dale & Dobell (1917) were the first workers to compare the virulence of *E. histolytica* from acute and carrier cases in kittens. They found that cysts from three carriers would not infect kittens while cysts from a fourth case infected, but the incubation period was twice as long as that observed with *E. histolytica* from acute cases. Cysts from carriers were also found to be non-infective to cats by Wagener & Thomson (1924), but the value of their experiments is reduced, since the size of the cats used apparently varied considerably (Dale & Dobell had previously found that kittens weighing between 500 and 600 g. were most suitable). Brumpt (1925, 1928) accepted the existence of avirulent strains of *E. histolytica* and proposed that a new species, *E. dispar*, should be created for them. Simic supported Brumpt's views and found that *E. histolytica* from carriers would infect kittens (1931) and dogs (1933) without producing ulceration.

A criticism of all this work is that no more than seven animals were used in each group nor was a standard method of inoculation used. This comment cannot apply to the series of papers by Meleney & Frye. These workers inoculated all their kittens by injecting the amoebae into the ileum, with at least twenty animals in each group. Their results showed that there was a definite difference in virulence between strains derived from two different geographical areas in Tennessee, U.S.A. (Meleney & Frye, 1933, 1935), though they found that strains from carriers gave infections as severe as those from acute cases (Meleney & Frye, 1933, 1935, 1937). The difference in virulence was maintained in two strains after 5 years cultivation *in vitro*, but another strain cultivated for a similar period lost virulence (Meleney, Frye & Leathers, 1939).

A group of Russian workers have made careful investigations into the effect of strains from different clinical types of amoebiasis. Ter-Matevossian, Sarkissian & Zaturian (1936) and Sarkissjan (1938) compared Armenian strains of *E. histolytica*, and concluded that the reaction of kittens to all of them was identical, irrespective of their origin from symptomless or acute cases. All strains successfully infected kittens causing dysentery, and ulceration was found at autopsy. As a result of further work, Matevossian, Sarkissian & Markaryan (1941) state that there may be slight differences in virulence between strains, but all cause ulceration in kittens.

Burova & Kakabadse (1941) studied three Caucasian strains in detail and found that two strains were less infective and virulent than a third strain from another geographical location. These authors reached a similar conclusion to Meleney & Frye in that local conditions may alter the virulence.

Kessel (1928), working in China, was unable to detect any difference between kitten infections produced by local strains of *E. histolytica* from symptomless and acute cases, and Faust, Heilbrunn, Lewis & Murray (1946) have recently published the results of their observations from which they concluded there is some strain variation. Unfortunately, their paper is so highly summarized it is not possible to

determine whether the virulence was correlated with the clinical history. Tobie (1940) concluded that all carrier strains produced ulceration since a total of twenty-six dogs were infected with sixteen strains of *E. histolytica*. All animals showed amoebic lesions at autopsy.*

These results are therefore somewhat contradictory. It seems likely that the inoculating material may be partly responsible for this variation. All those workers using culture amoebae describe the uniform infection of kittens with the production of ulceration, while the results of those using cysts are variable. But even with that factor eliminated, analysis of the foregoing observations reveals small but constant differences in the results of infection with strains of different clinical types. In addition, this work provides abundant evidence that kittens are very susceptible to infection with *E. histolytica* resulting in ulceration.

We believe that the reason for the disparity between rats and kittens in their reaction to infection with *E. histolytica* from human cases of different clinical types, lies, not in the masking of the invasive properties in carriers as the kitten experiments suggest, but in the singular susceptibility of the feline laboratory host. The hypersensitivity of the kitten is confirmed by experiments with simian strains of *E. histolytica*. It is generally agreed that ulceration of the large intestine caused by this amoeba is rarely observed in monkeys of the genus *Macaca*,† yet kittens inoculated with *E. histolytica* from macaques, suffer from amoebic dysentery almost indistinguishable from that caused by human strains (Kessel, 1928; Dobell 1931; Burova, 1939).

A paper has been published recently by Okamoto (1953) comparing the degree of invasiveness of *E. histolytica* in rats with that observed in man. It seems at first sight that Okamoto's results confirm those published previously (Neal, 1951*a*) and agree with the above experiments, but two reservations concerning his experiments must be made. These are that cysts from human faeces were used as the inoculum and that the rats were relatively large—average body weight varying from 41·2 to 77·8 g. It is well known that the use of an unstandardized inoculum of cysts gives unpredictable results, while large rats weighing more than 30–35 g. are less susceptible to infection with *E. histolytica* than young rats, the infection rate and the degree of ulceration being reduced (Jones, 1946). Although his experiments cannot be regarded as convincing, it is interesting to note that Okamoto also concluded that carrier strains are non-invasive to rats.

Other evidence purporting to prove that *E. histolytica* always invades the intestinal wall is derived from material obtained from human autopsies. This whole question has been extensively reviewed by Hoare (1950, 1952). The absence of ulceration in the caecum and colon of the rats examined by sectioning shows that *E. histolytica* can live as a commensal and adds further evidence that human carriers

* This paper is rather difficult to understand, since although the author arrives at the conclusion quoted above, he also states that half of the experimental animals (13 in number) did not suffer from dysentery, and at autopsy they were free from amoebae though some lesions were observed. The evidence for the amoebic origin of these lesions is not given.

† This discussion is limited to *Macaca* since most work has been carried out with this genus, and Miller (1952) has suggested that New World monkeys may react differently from Old World monkeys in regard to ulceration by *E. histolytica*.

of *E. histolytica* may not suffer from intestinal ulceration. In spite of careful microscopical examination, no minute lesions of the type observed by Bond, Bostick, Hansen & Anderson (1946) in the large intestine of macaques and by Johnson (1941) in *Ateles geoffroyi*, *A. dariensis* and *Macaca rhesus* were seen. It should be noted that in Miller's opinion (1952) the lesions seen by Bond *et al.* were due to previous drug treatment.

In a previous study (Neal, 1951*a*) it was concluded that non-invasive strains were more susceptible to treatment with emetine hydrochloride than invasive strains, and that all strains of the former type were completely susceptible to six daily oral doses of 2·0 mg./kg. In the present work, however, one strain (*HK*, no. 10) was not affected by 2 mg./kg., although susceptible to 4 mg./kg., while another (*ZM*, no. 2) was partly resistant to 4 mg./kg. This shows that the effective dose of emetine may vary from 1 to more than 4 mg./kg. and reopens the question as to whether the amount of drug required for effective treatment is greater or smaller in the presence of ulceration. Unfortunately, these 'resistant' non-invasive strains were not treated with any other amoebicidal drug. Jones (1947) reported that a course of 2·5 mg./kg × 5 was required to cure his strain of *E. histolytica* and on the basis of the previous results (Neal, 1951*a*) it was suggested that the presence of *E. muris* may have confused the results of Jones's experiments. In the light of the further experiments described above, it is obvious this comment cannot be justified.

The use of rats as experimental animals for the study of amoebiasis has been criticized on the grounds that they may only be used for the reproduction of the acute disease. However, the above experiments confirm that an infection without ulceration is also produced in this host. Examination of the capacity of *E. histolytica* to produce ulceration in the various natural or experimental hosts shows that macaques are most resistant to ulceration by *E. histolytica* (see Miller, 1952), kittens most susceptible, while rats and man fall between these two extremes.

We realize that human infections can rarely be definitely classified as either carrier or acute diseases; there are a series of intermediate types from one extreme to the other. However, we selected the extremes for investigation in order to make the experimental results as precise as possible.

In the light of present experiments, it can be seen that there is a definite difference between the cultures derived from carriers and those from acute cases, though it is not yet known beyond all doubt whether this difference is due to the protozoan or bacterial components of the culture. In addition, it should not be concluded that strains isolated from a particular case always remain invasive or non-invasive. In view of the frequent occurrence of relapses in amoebiasis, it is possible that the invasiveness to rats might vary with the clinical condition of the host.

SUMMARY

1. Eleven strains of *Entamoeba histolytica* were isolated from contact carriers and all proved non-invasive to rats.

2. Three strains from cases showing symptoms proved invasive to rats.

3. The treatment of murine infection with non-invasive strains with emetine hydrochloride shows that most of them were successfully cured with six daily oral doses of 2 mg./kg., though one was not susceptible to this dose. Another proved refractory to 4 mg./kg.

4. The results of previous work with kittens are discussed in the light of the present observations.

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PROBLEMS CONCERNED WITH GAMETOGENESIS IN HAEMOSPORIDIIDEA, WITH PARTICULAR REFERENCE TO THE GENUS *PLASMODIUM*

By ANN BISHOP*

Molteno Institute, University of Cambridge

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I. INTRODUCTION

Our knowledge of the morphology and significance of the gametocytes of the parasite causing malaria was derived not only from work upon the genus *Plasmodium*, but from observations upon other members of the Haemosporidiidea. Many observations upon gametocytes have been made coincidentally with work upon other problems, and the following account is an attempt to collect together some of the data upon their development and the factors which may affect their production.

II. HISTORICAL

It was his observation of exflagellation in the blood of a patient suffering from malaria—an observation first made on 6 November 1880—which led Laveran (1881) to conclude that the pathological condition was due to the presence of a parasitic organism in the blood. Five years later, similar observations were made by Danilewsky (1885) upon a haematozoan, *Haemoproteus*, which he found in the blood of birds. He described the emergence of the organism from the red blood corpuscles and the rotatory movement caused by the flagella. Later (Danilewsky, 1889) he gave it the name of *Polimitus sanguinis avium*, and he remarked upon its similarity to the parasite observed by Laveran. Neither he nor Laveran understood

* Member of the Scientific Staff, Medical Research Council.

the biological significance of the flagellating bodies which they described so accurately, though Laveran noted the separation of the flagella from the central mass.

In 1893, Sakharoff, using the recently discovered Romanowsky stain, demonstrated that the filaments of the flagellating bodies were chromatic.

Manson (1894) believed that the formation of the flagellating bodies was an integral part of the development of the parasite essential for the life of the species outside the body of the host, and not, as suggested by some of his contemporaries, a degenerative process due to the abnormal conditions in the drawn blood. By analogy with his observations on filaria, Manson suggested that the parasite was removed from the blood by a mosquito or other suctorial insect and that the flagellated organism must pass into some organ of its body. This hypothesis was supported by the observation made by Ross, on 25 May 1895 (Manson, 1896), that exflagellation occurred in the stomach of mosquitoes reared from the egg and fed upon a patient heavily infected with crescents of malignant tertian malaria.

In a species of *Halteridium*, Labbé (= *Haemoproteus*) Opie (1898) differentiated between deeply stained granular parasites of the red blood corpuscles with small nuclear-like areas, and palely staining forms with large vesicular areas. He observed that both types of organism became free from the red blood corpuscles. He raised the question whether both developed flagella, and was inclined to believe that this change occurred only in the swollen palely staining forms, but he was unable to verify this opinion experimentally. MacCallum (1898) confirmed Opie's description of the two types of parasite and showed experimentally that it was the pale forms which became flagellated; moreover, he interpreted the biological significance of exflagellation. He observed one of the flagella, which had torn itself free from the central mass, fuse with one of the quiescent granular parasites and become absorbed by it. After a period of about 15 min., the sphere changed into a fusiform body which glided away. This elongated organism corresponded closely to the vermiculi first described by Danilewsky (1889) in the blood of birds and which he had seen develop from extracorpuscular spherical bodies, though he had not seen fertilization take place. MacCallum realized that what he had seen was a sexual process giving rise to a motile zygote; but the future development of the zygote remained a mystery to him, though he suggested that it might be a resistant stage which penetrated to the intestine and so to the outside world. He also saw exflagellation, and fertilization of the female gamete, in blood from a woman suffering from malignant tertian fever, but, though crescents were numerous, vermicules, i.e. oökinetes, were not formed on the slide as they had been in *Haemoproteus*.

Before MacCallum solved the problem of the significance of exflagellation, Ross (1897) had discovered spherical bodies, containing pigment, on the stomach wall of two 'dapple winged' mosquitoes killed on the 4th and 5th day after being fed on a patient whose blood contained crescents. Later, he (Ross, 1898*a*) also found similar bodies on the wall of the stomach of 178 out of 245 'grey' mosquitoes fed upon birds infected with *Proteosoma*, and was able to follow the development of these cysts to the production of 'germinal rods', i.e. sporozoites, which he traced to the salivary glands (Manson, 1898; Ross, 1898*b*, *c*). Moreover, Ross (1898*c*) performed

the crucial experiment of transmitting the infection to sparrows and a weaver bird, which observation had shown to be free of infection, by the bite of mosquitoes which had fed, a week previously, on a sparrow whose blood contained the organism. Ross's work was confirmed by Koch (1899*a*, *b*) who, in addition, described oökinetes in the stomachs of mosquitoes which, 12–15 hr. previously, had fed upon birds infected with *Proteosoma*. Although he did not actually observe their penetration through the stomach wall, Koch noticed that the oökinetes began to disappear from the contents of the stomach after 48 hr. whilst, simultaneously, small spherical bodies containing pigment granules appeared on the stomach wall. It was Grassi (1900) who, in working out the development of the parasites of benign and malignant tertian malaria in mosquitoes, demonstrated the presence of oökinetes in sections of the stomach wall. Our knowledge of the development of the gametocytes of *Plasmodium* in mosquitoes was thus completed.

III. GAMETOGENESIS IN *PLASMODIUM* AND THE PROBLEM OF A REDUCTION DIVISION

Although the asexual parasites produce the clinical symptoms known as malaria, the gametocytes, alone, effect the distribution of the disease; but in spite of their great importance little is known of their mode of development. The theory generally accepted is that they are produced from merozoites as a result of an unknown stimulus. Both macro- and microgametocytes have been observed in the initial infections derived from a single asexual parasite of *P. gallinaceum* (Downs, 1947), an observation confirmed by the writer; but whether both sexes arise from the same schizont is not known. Boyd (1935) described five types of erythrocytic parasite in *P. vivax*; one type, the schizogony-producing cells, being the stem cells from which the other four types, consisting of pregametocytes and gametocytes, develop. Corradetti (1936), however, was unable to confirm these observations. He described two types of rosette in *P. vivax* which differed in the arrangement of the pigment and merozoites, and in the nuclear and cytoplasmic characters of the merozoites themselves. He suggested that they may correspond to the two methods of segmentation described by Golgi in 1889, and that one type may give rise to further generations of schizonts, whereas the other may be destined to produce gametocytes.

Observations upon avian malaria have suggested that gametocytes may also arise directly from exoerythrocytic schizonts. Adler & Tchernomoretz (1943) inhibited the development of erythrocytic parasites with quinine in chickens infected with sporozoites of *P. gallinaceum*. When large numbers of uninucleate merozoites, arising from the exoerythrocytic schizonts, were observed in the erythrocytes, drug treatment was stopped, and the blood examined up to and including the first schizogonic division in the erythrocytes. Gametocytes were distinctly recognizable 27½ hr. after the last dose of drug, and after 33 hr. the largest occupied more than two-thirds of the erythrocyte in which it lay, whereas the first mature schizont was not found until 34 hr. after the last dose of drug. It must be concluded, therefore, that the gametocytes present during this period had arisen directly from the exoerythrocytic schizonts.

Further evidence of the relationship of gametocytes to exoerythrocytic parasites comes from some work of Lewert (1950). A strain of *P. gallinaceum*, which was derived from exoerythrocytic parasites grown in tissue culture, was maintained in young chicks by serial passage of brain tissue heavily infected with these forms. In the resulting infections merozoites arising from the exoerythrocytic parasites penetrated into the erythrocytes but did not develop further. Blood from such birds, when inoculated into uninfected chickens, produced infections in which the proportion of gametocytes to asexual parasites was very high, though in the original strain from which the tissue cultures were derived, few gametocytes occurred. Although in these experiments there is no direct evidence that the gametocytes arose from exoerythrocytic schizonts, the maintenance of the strain for a long period in the exoerythrocytic phase appears to have stimulated the production of these forms.

Whereas the nuclei of the asexual parasites of several species of *Plasmodium*, when exposed to the Feulgen nucleal reaction for the detection of desoxyribonucleic acid, have been found to give a positive reaction, which varied in intensity with the stage of development of the parasite and the species, the nucleus of the gametocytes either failed to stain or did so only faintly (Jírovec & Černý, 1932; Chen, 1944; Deane, 1945; Lewert, 1952; Bishop, 1954). An explanation of this difference in reaction between the nuclei of the asexual parasites and the gametocytes is suggested by recent work upon the nuclei of vertebrates. It has been shown that the amount of DNA present in somatic cells is constant within a species, the haploid sperm nucleus containing half the amount present in a somatic cell (Boivin, Vendrely & Vendrely, 1948; Vendrely & Vendrely, 1949, 1950). If these observations hold good for the nuclei of *Plasmodium*, the amount of DNA distributed through the large nucleus of a gametocyte will be equivalent to that in the condensed nucleus of a single merozoite, and the concentration may thus be too low for detection by the Feulgen method.

Our knowledge of the changes which take place in the nucleus of the gametocyte of *Plasmodium* during gamete formation and fertilization is fragmentary, the small size of the parasite making such a study a difficult one. Early observers assumed that a reduction division occurred during the development of the gametes from the gametocytes. Bastianelli & Bignami (1900*a*) described minute hyaline spherical bodies, containing chromatin, on the surface of the macrogametocytes of *P. falciparum*, and suggested that they might be reduction bodies (Bastianelli & Bignami, 1900*b*). According to Bastianelli & Bignami these bodies were first observed by Machiafava and Celli. Schaudinn (1902) described the formation of spherical bodies, containing nuclear material, on the surface of the macrogametocytes in blood from the stomach of mosquitoes which had fed, 10–20 min. previously, upon patients infected with *P. vivax*; like the Italian workers, he believed them to be reduction bodies. He also observed that, prior to the formation of the microgametes, the microgametocytes contained eight nuclei, though the number of microgametes produced varied from four to eight.

Since these early descriptions, important observations have been made upon the nuclear changes occurring during gametogenesis and fertilization in other members

of the Sporozoa. The work of Dobell (1925) upon the coccidian *Aggregata eberthi* and of Jameson (1920) upon the gregarine *Diplocystis schneideri*, a preliminary account of which was published in 1915 (Dobell & Jameson, 1915), has shown that, with the exception of the zygote which, prior to the first nuclear division, has a diploid number of chromosomes, these organisms are haploid throughout their life cycle. In both organisms the reduction division is zygotic not gametic. Evidence of a similar chromosome cycle has also been found in the haemococcidian *Karyolysus* (Reichenow, 1921); in *Klossia helicina* (Naville, 1927*a*), *K. loosi* (Nabih, 1938) and *Adelina cryptocerci* (Yarwood, 1937), members of the Adeleidae Léger; in *Merocystis kathae* (Patten, 1935), a member of the Aggregatidae Labbé; in *Barrouxia schneideri* (Wedekind, 1927) belonging to the Eimeriidae Léger; and in the schizogregarine *Mattesia dispora* (Naville, 1930). In Eugregarines, however, both zygotic and gametic reduction divisions have been described. In *Monocystis rostrata* the reduction division is, according to Mulsow (1911), gametic, an observation confirmed by Calkins & Bowling (1926), and by Naville (1927*b*) who studied the chromosome cycle in three species of this genus; more recently, however, Cleveland (1949) has referred to unpublished work of Negherbon in which the division is described as zygotic. In *Urospora lagidis* Naville (1927*c*) found that reduction of the chromosomes occurred in the gametes. Zygotic reduction divisions have been described in *Monocystella arndti* (Valkanov, 1935), in *Apolocystis elongata* (Phillips & Mackinnon, 1946) and in *Actinocephalus parvus* (Weschenfelder, 1938); moreover, Guyénot & Ponse (1926) have found evidence of a haploid cycle in the microsporidian *Plistophora bufonis*, since three grains of chromatin were found in 354 out of 360 nuclear divisions during microsporogony.

MacDougall (1947) has studied the cytological changes occurring in the male gametocyte of *Plasmodium falciparum* and *P. vivax* during the formation of the gametes, but the sequence of the developmental stages observed was not complete. In the earliest stage, the granules in the nucleus appeared to be arranged in threads which were believed to correspond to a leptotene stage. Later, four parallel rows of granules were observed which, it was suggested, corresponded to a zygotene stage which had arisen by pairing of the thinner leptotene threads. Of these four paired chromosomes, one pair was heterotypic. A pachytene stage followed in which the four pairs of chromosomes appeared to be fused into a single thick thread which showed the line of parasynapsis throughout its length. This thick thread was believed to give rise to four pairs of chromosomes again later. After the flagella had developed, one paired mass of chromatin was seen to enter each microgamete, but whereas the number of flagella formed was four to eight, the number of paired chromatin masses appeared to be four. It was observed that some male gametes which broke off from the residual mass were devoid of a nuclear element and were thus sterile. The relationship of the paired chromatic structures entering the male gametes to the four pairs of chromosomes, which were believed to arise from the pachytene stage, is obscure, nor is any light thrown upon the stage at which the reduction division takes place.

Lüdicke & Piekarski (1952) have studied the nuclear changes occurring during the formation of the gametes and fertilization in *P. falciparum*. According to these

workers, nuclear division in the male gametocyte is endomitotic. A scattered chromatin network was first formed from which paired chromosome-like elements, which were interpreted as homologous pairs, arose, and which were believed to correspond to sets of chromosomes rather than to individual chromosomes. In some preparations twelve nuclei were visible prior to the formation of the microgametes, and were thought to correspond to the six homologous pairs. The number of gametes produced varied from four to twelve. A Feulgen-positive residual body sometimes remained after the microgametes had become free, but Lüdicke & Piekarski did not regard it as a reduction body.

In the macrogametocytes Lüdicke and Piekarski describe three or four pairs of thick chromatin structures lying parallel to each other, in many cases one pair being smaller than the others. Maturation bodies, containing Feulgen-positive material, were formed on the surface of the macrogametocytes, as described by earlier workers, and since, in a large proportion of macrogametocytes, these bodies were formed before the male gametes had developed, they must have arisen before fertilization had taken place. Lüdicke & Piekarski believed them to be reduction bodies.

Although a nuclear division with chromosome reduction was never actually seen, Lüdicke & Piekarski conclude that the presence of the paired chromosome-like structures, and the formation of reduction bodies in the female gametocytes prior to fertilization, are evidence of a pre-gametic reduction division giving rise to haploid gametes.

The formation of Feulgen-positive maturation bodies in the macrogametocyte of the malaria parasite before fertilization cannot be accepted unreservedly as evidence of a pre-gametic reduction division. 'Polar' nuclei, assumed to be reduction bodies, were described by Paehler (1904), Schnitzler (1905) and Léger & Duboscq (1909) in the gametes of several species of gregarines, but Dobell & Gameson (1915) pointed out that no reduction in the number of chromosomes was demonstrated during their formation, and the fact that the chromosome numbers, in the two species, *Nina gracilis* (Léger & Duboscq, 1909) and *Clepsidrina ovata* (Schnitzler, 1905), in which they could be counted, appeared to be odd made a reduction division at that stage improbable. The function of these bodies therefore is enigmatic.

In this connexion an observation by Naville (1930) upon the schizogregarine *Mattesia dispora* is of interest. During all stages of the developmental cycle of this organism, with the exception of the zygotic nucleus which prior to its first division had four chromosomes, the nuclei each contained two chromosomes. Two binucleate residual bodies each containing the haploid number of chromosomes were observed in the cysts at the time of the formation of the gametes. Since these bodies were formed before copulation of the gametes had taken place, and since the reduction division was zygotic, they could not be reduction bodies.

In comparing the nuclear changes occurring in the microgametocyte of *P. falciparum* as described by MacDougall and Lüdicke & Piekarski, although paired chromosome-like structures were described by both the American and German workers, the accounts differ in other details. It is obvious that in such a small organism the nuclear changes must be very difficult to follow and even more diffi-

cult to interpret, but until the reduction division has been seen and described it is impossible to decide whether the malaria parasite, like most other members of the Sporozoa whose chromosome-cycle is known, is haploid, or whether it is diploid.

IV. THE EFFECT OF THE PHYSIOLOGICAL CONDITION OF THE HOST UPON THE NUMBERS OF GAMETOCYTES PRODUCED

Evidence of a direct effect of environmental factors upon the sexual cycle of Protozoa is accumulating. It has long been known that in the Opalinidae the sexual phase of the life cycle occurs in spring, the reproductive season of the amphibian host. Lwoff & Valentini (1948) have found that in a pure culture of *Cepedea dimidiata* growing in a medium containing frog's liver, cysts appeared in large numbers during the months of April and May, and they suggested that the liver might, at that season, contain a hormone which stimulates encystment. According to Bieniarz (1950), the injection of pregnancy urine into frogs caused the encystment of Protozoa in the rectum, though similar results were not obtained *in vitro*. Bieniarz considers that the change from asexual to sexual reproduction in these Protozoa, marked by the development of cysts, may be stimulated by human chorionic gonadotropin present in the pregnancy urine. In Protozoa inhabiting the hind-gut of the wood-feeding roach, *Cryptocercus punctulatus*, Cleveland (1949) has found that the sexual cycles are controlled by a hormone which causes the host to moult, since any treatment which slows up or stops moulting affects the sexual cycles of the Protozoa in the same way.

Observations have been made upon *Leucocytozoon simondi* and *Haemoproteus columbae* in wild and domestic ducks which show that in these haemosporidia schizogony and gametocyte production are affected by the physiological condition of the host. In mallard ducks (*Anas p. platyrhynchos*) the number of gametocytes present in the blood increased rapidly in both species of parasite during the months of March and April (Huff, 1942). As it was too early in the year for this increase in number to be attributed to new infections transmitted by the bite of *Simulium*, Huff considered that the most probable explanation was that some relapse mechanism was involved which set into action the schizogonic process producing the gametocytes, and he suggested that it might 'be correlated with the physiological ossification of bone marrow antecedent to egg laying, with the accompanying removal of myelocytopoietic cells from the tissue.'

Chernin (1952) has studied the relapse phenomenon in *Leucocytozoon simondi* over a period of 3 years, and has found that the increase in number of gametocytes in the blood stream is associated with the onset of reproductive activity in the host and occurs in both sexes. By exposing female birds to increased hours of artificial light each day during the autumn and winter months, the commencement of the period of egg laying could be hastened by several weeks or months, and the time of increased gametocyte production hastened likewise. There was no evidence that light *per se* had any effect upon the numbers of gametocytes produced except in its effect upon the reproductive activity of the host. Thus some birds which had been exposed to increased hours of light over a long period, failed to undergo a change in reproductive rhythm, and in these birds no early relapse of the parasitic

infection occurred. Moreover, it was necessary for birds to have reached a certain degree of maturity before an increase in exposure to light affected either their time of egg-laying or the time of the increased production of gametocytes. Chernin points out that ossification of the bone marrow does not occur in drakes, whereas the increase in gametocyte-production occurs in this sex simultaneously with the females. He considers that a stimulation of the anterior pituitary is probably involved in the relapse phenomenon, since it has been shown to be the essential organ concerned in gonad stimulation by light.

That the season of the year may affect the numbers of gametocytes produced in acute infections of *Plasmodium relictum*, induced in canaries by blood-inoculation, was suggested by some observations made over a period of 2 years by the writer (Bishop, 1943). The number of gametocytes produced was greater from April until July or August, than at other times of the year. In both years the proportion of gametocytes to asexual parasites dropped sharply during the autumn months and rose again sharply in the spring.

V. THE RELATIONSHIP OF THE NUMBERS OF GAMETOCYTES PRODUCED TO THE TYPE OF HOST IN WHICH A STRAIN IS MAINTAINED

The type of host in which a strain of *Plasmodium* is maintained may affect the numbers of gametocytes produced. Infections of *P. mexicanum* in the blood of the natural host, the lizard *Scoloporus ferrariperezi*, consists primarily of gametocytes, but when the parasite was transmitted to twelve specimens of *Crotaphytus collaris* no gametocytes were observed over a period of a year, though they appeared in varying numbers when the strain was transmitted from *C. collaris* to four other species of lizard (Thompson & Huff, 1944).

When howler monkeys, *Alouatta* sp., were infected with *Plasmodium falciparum*, only sexual parasites were found, although in one animal the infection reached a peak of 915 parasites per 10,000 red cells (Taliaferro & Taliaferro, 1934).

More recently, McGhee (1951) has established infections of *P. lophurae*, of low-grade parasitaemia, in infant mice. Gametocytes were absent from all infections with the exception of one mouse in whose blood two doubtful specimens were seen. The fact that gametocytes were numerous in chick embryos infected with the strain, either in its early passages through baby mice, or after it had become adapted to its new host, proved that the lack of gametocytes was due to the change of host and was not an intrinsic characteristic of the strain.

Strains of *P. berghei* isolated from a tree rat (*Thamnomys surdaster surdaster*) of the Belgian Congo (Vincke & Lips, 1948) may lose the ability to form gametocytes when passaged through other small mammals by blood-inoculation, but whether this is due to maintenance in unnatural hosts or to an unnatural method of transmission is not clear.

Although a change in the numbers of gametocytes produced by a strain may thus be associated, in some instances, with maintenance in an unnatural host, in others it appears to be inherent in the strain itself. Thus *Plasmodium nucleophilum* failed to produce gametocytes when inoculated into ducks, but it also ceased to produce them in canaries, the host from which the duck strain was derived

(Manwell, 1943). A strain of *P. elongatum* originally maintained in canaries, ceased to produce gametocytes after fifteen passages through ducks (Micks, 1947); but a strain derived from the duck strain before it ceased to produce gametocytes and maintained in canaries, likewise ceased to produce them, whereas the original strain, maintained in canaries, which may be considered to be an unnatural host since *P. elongatum* was isolated from an English sparrow, continued to produce up to 90 % gametocytes at the height of parasitaemia. The inoculation of the gametocyteless duck strain into the natural host—the English sparrow—failed to stimulate gametocyte production, nor was this procedure successful with a gametocyteless strain of *P. cathemerium* (Gambrell, 1937). It might be assumed that its sojourn in the duck had so affected the strain of *P. elongatum* that it had lost its power to produce gametocytes even when transferred to canaries, but a strain maintained in ducks by Wolfson (1946) through more than forty serial passages still produced them. In none of these cases is there any clear evidence that the change in gametocyte production was related to the type of host in which the strain was maintained.

VI. THE EFFECT OF THE METHOD OF TRANSMISSION UPON THE NUMBERS OF GAMETOCYTES PRODUCED

It is well established that the maintenance of a strain of *Plasmodium* by blood-inoculation over a long period, may affect the numbers of gametocytes produced. Soon after malaria therapy was introduced for the treatment of general paralysis of the insane, it was observed that in some strains of *P. vivax* which had been maintained by the inoculation of infected blood, gametocytes could no longer be found (Barzilai-Vivaldi & Kauders, 1924; Schulze, 1925), and attempts to transmit one of these strains through mosquitoes was unsuccessful (Barzilai-Vivaldi & Kauders, 1924). In Wagner Jauregg's clinic two strains of *P. vivax*, maintained by blood-inoculation, which had originally produced gametocytes though in small numbers, ceased to produce them (Cuboni, 1926). Loss of the ability to produce gametocytes was not, however, inevitable in strains maintained in this way since Mühlens & Kirschbaum (1924) found gametocytes in strains maintained by blood-inoculation over a period of 5 years.

It has been shown that the numbers of gametocytes produced by a strain may fall suddenly or gradually (Boyd, 1945), and that the ability to produce them may not be lost simultaneously in all substrains of a parent strain (Jeffery, 1951).

Similar changes in gametocyte production to those observed in human malaria have been seen in strains of avian malaria maintained by blood-inoculation. A strain of *P. cathemerium* maintained in canaries suddenly ceased to produce gametocytes, and the passage of large inocula at frequent intervals failed to restore it to its former condition; but a substrain, recovered from a bird with a latent infection of the original strain before it ceased to form gametocytes, produced them in large numbers (Huff & Gambrell, 1934). Subinoculations, made from strains kept in a state of latent infection for years, produced large numbers of gametocytes, provided that the strain, at the time when it became latent, was producing large numbers of gametocytes. The latent state did not itself increase the ability of a strain to form gametocytes, since latent infections of the gametocyteless strain did

not give rise to infections with gametocytes—it merely preserved the character of the strain (Gambrell, 1937). That a latent period does not always act in this way was suggested by observations made by Boyd (1945) upon a strain of *P. falciparum* in which a marked fall in the numbers of gametocytes was first observed in sub-inoculations from donors with clinically latent infections.

The loss of the ability to form gametocytes may be a very stable character since the gametocyteless strain of *P. cathemerium* still failed to form gametocytes after a period of 12 years (Huff & Coulston, 1946). Plehn (1925), however, reported that a strain of *P. vivax* suddenly began to produce gametocytes after a period of 2 years during which it had been passaged twenty times without gametocytes being observed.

Since there is evidence that gametocytes can arise from exoerythrocytic parasites, it might be expected that an increase in their number would occur, in the primary infection at least, if a strain poor in gametocytes were transmitted through mosquitoes, but the experimental evidence is conflicting. Gambrell (1937) found no evidence that mosquito transmission increased the number of gametocytes in strains of low gametocyte production, but, owing to the difficulty in infecting mosquitoes with such strains, it was not possible to study the effect of repeated mosquito transmission. The effect of mosquito transmission was found to differ between two strains derived from a single clone of *P. cathemerium*; thus a strain which had become progressively poorer in gametocytes whilst being maintained by blood-inoculation, regained its ability to produce them in large numbers after being passed through mosquitoes, whereas a second strain, also derived by mosquito transmission from the same clone, failed to regain this character (Huff, 1941).

Workers upon *P. gallinaceum* have found that, whereas in infections maintained for long periods by blood inoculation the numbers of gametocytes produced may decrease and difficulty may be experienced in infecting the insect vector, *Aëdes aegypti*, in strains maintained wholly by mosquitoes large numbers of gametocytes continue to be produced and no difficulty is experienced in infecting mosquitoes. During the therapeutic use of malaria in the treatment of general paralysis of the insane, James, Nicol & Shute (1936) observed an increase in gametocyte production in a strain of *Plasmodium vivax* transmitted by mosquito. When the Madagascar strain was first introduced into their laboratory, James and his colleagues found that although gametocytes might appear from the sixth day of the primary attack, they were seldom present in numbers sufficient to produce infection in mosquitoes before the tenth day; later, however, after further passages through the insect and human host, gametocytes appeared on the third day of the primary attack and were present in greater numbers. This increase in the number of gametocytes was reflected in the oocyst count; whereas in early feeding experiments the number of oocysts per stomach rarely exceeded ten, in later experiments, when gametocyte numbers had increased, it was often as great as several hundreds.

It has been suggested that long residence in the vertebrate host, with constant asexual reproduction, enhances the asexual character of the strain (Gambrell, 1937). The mosquito presumably acts as a selective agent, since only virile gameto-

cytes well-adapted to it would complete their development in that host. Any tendency of parasites within a strain to produce larger numbers of gametocytes would be of selective value, provided that they were infective, and a mosquito-transmitted strain would, therefore, tend to be composed of asexual parasites with the greatest potentialities for gametocyte production. Such a tendency would not be of selective value in syringe-transmitted strains where the emphasis would be on characters which fitted the parasite for survival in the vertebrate host.

The possibility that changes in gametocyte production may arise by mutation must also be considered. A mutation leading to an increase in the number of gametocytes, provided that they were of normal infectivity, would be of selective value in mosquito-transmitted strains but not in those maintained by blood-inoculation. A mutation towards the production of fewer gametocytes in a strain maintained by blood-inoculation would presumably be associated with the production of a larger number of asexual parasites which might lead to the overgrowth of strains producing greater numbers of gametocytes. The sudden appearance of gametocyteless strains, as recorded by several workers, however, seems hardly possible by such a means. The gametocyteless condition described by Huff & Gambrell (1934) in a strain of *P. cathemerium* developed suddenly, but the fact that it had a smaller number of merozoites per segmenter than the normal strain from which it was derived (Gambrell, 1937) is an argument against its having arisen by selection.

A decline in the ability of the malaria parasite to form gametocytes has been put forward by Sautet (1953) to account for 'spontaneous regression' of the disease. He supports his argument by observations based on three zones in Guadeloupe: in one area, which was very malarious, the gametocyte index was high; in a second area where the incidence of malaria was lower, the gametocyte index was low; and in a third zone from which malaria had almost completely disappeared, no gametocytes were found. It must be presumed that, in an area where the decline in incidence of malaria was due to this cause alone, the accidental introduction of a strain of parasite producing large numbers of gametocytes would lead to an increase in incidence, since all the factors necessary for transmission would be unchanged.

VII. THE RELATIONSHIP OF THE NUMBER OF GAMETOCYTES PRODUCED TO THE STATE OF IMMUNITY IN THE HOST

Early workers tended to attribute the development of gametocytes to the effect of immunity, but later surveys have shown that these forms are more prevalent in acute than chronic infections. Thus Christophers (1924) found that in infections of *P. falciparum* gametocytes were most numerous during the period of acute infection and when the infections were most intense. A distinct correlation between the numerical prevalence of asexual parasites in the peripheral circulation and the number of crescents which appeared about 10 days later was observed by Sinton, Baily & Chaud (1926). In primary infections of *P. cathemerium* in canaries Huff (1927) found that the numbers of gametocytes increased with the increase in total number of parasites, the peak in the number of gametocytes being 1 or 2 days later than that of the total parasitaemia; during relapse the proportion of gametocytes

to asexual parasites was lower than in primary infections. An increase in number of gametocytes with the increase in total parasite count was observed in blood-inoculated infections of this species by Shah (1934) also, but, unlike Huff, he observed a periodic increase in their number at the time of sporulation. A well-marked rise in the proportion of gametocytes to asexual parasites as schizogony approached was also observed in *P. gallinaceum* (Lumsden & Bertram, 1940).

The production of gametocytes in human malaria does not appear to be a uniformly continuous process; thus in infections with *P. falciparum* Garnham (1931) has described the sudden flooding of the blood with crescents, and in *P. vivax* the appearance of 'showers' of gametocytes after the completion of a fairly definite number of schizogonic divisions has also been described (Boyd, Stratman-Thomas & Muench, 1936).

In experimentally induced infections of *P. vivax* (Boyd, Stratman-Thomas & Muench, 1936) gametocytes have been found on the first day on which parasites were detected in the blood, and similar observations have been made in *P. cathemerium* (Shah, 1934) and *P. gallinaceum* (Lumsden & Bertram, 1940; Eyles, 1951; Bishop, unpublished observations), whilst in *P. relictum* infections in canaries they were sometimes seen before asexual parasites could be found (Bishop, 1943). Acquired immunity could scarcely play any part in their production at such an early stage of the infection.

An inverse relationship between the numbers of gametocytes produced and the development of immunity was demonstrated in a survey made by Schüffner (1919, 1938) in the Dutch East Indies. In untreated, primary infections of malignant tertian malaria crescents were usually present, in variable numbers, for about 3 months. Their incidence was high in areas where the introduction of the disease was recent and low or absent where it was of long standing. Crescents were numerous in children too young to have developed immunity to the disease, their number decreasing with advance in age. In an area where malaria had been established for several generations and transmission occurred all the year round, 51 % of the children in the first 2 years of life were crescent carriers, whereas only 8 % of infected adults harboured crescents in their blood. In contrast to this, in an area in which malaria was epidemic, carriers of crescents were numerous in all the age groups. These observations suggested that with advancing age and under the development of immunity, *P. falciparum* gradually lost its capacity for producing gametocytes.

The greater frequency of crescents in young children was confirmed by Swellengrebel & Swellengrebel (1920) who found that the number of sexual parasites decreased more rapidly than that of the asexual parasites with increasing age of the host. These authors suggested that the production of crescents is either correlated with the absence of immunity or, if immunity does play some part in their production, its effect must be confined to the early stages before the fall in the intensity of infection or in the spleen rate is discernible. Wilson (1936), working in Tanganyika, observed that gametocytes were usually present in the second year of life, during the period when the child was passing from a state of acute infection into an immune state, but they became increasingly uncommon after the fifth year. The greater

incidence of crescents in young children has been confirmed by workers in other parts of the world, e.g. Bruce Chwatt (1951) in Nigeria and Muirhead-Thomson (1954*b*) in West Africa.

VIII. THE INFECTIVITY OF GAMETOCYTES TO MOSQUITOES

A relation between the number of susceptible mosquitoes infected and the numerical density of gametocytes in the blood has been observed in several species of malaria parasite and mosquitoes. Shah, Rozeboom & Rosario (1934) found that in *Culex pipiens* fed on canaries infected with *Plasmodium cathemerium*, the rate of infection increased with the numbers of gametocytes ingested until, when the number of gametocytes was as great as 10,000–50,000, 100 % became infected. In *Aëdes aegypti* both the proportion of mosquitoes infected and the oocyst count per stomach rose with increasing numbers of gametocytes of *Plasmodium gallinaceum* ingested (Lumsden & Bertram, 1940); in batches in which the average count exceeded fifty oocysts per stomach no negative mosquitoes were found. In *Anopheles stephensi* the percentage of mosquitoes infected with *Plasmodium vivax* or *P. falciparum*, with some marked exceptions, increased with an increase in the number of gametocytes in the patients' blood (Knowles & Basu, 1943). More recently, a similar relation has been found between *P. falciparum* and *Anopheles gambiae gambiae* (Draper, 1953).

In spite of this broad correlation between density of gametocytes and oocyst rate and density many investigators have found that some hosts are good infectors of mosquitoes, whereas others are not. This difference in infectivity is independent of the differences in susceptibility which exists between individuals in a batch of mosquitoes. James (1931) found that a high gametocyte count did not necessarily insure high rates of infectivity in *Anopheles*; this was particularly so in infections of *Plasmodium falciparum* and *P. malariae*, though it also occurred in *P. vivax*. He considered that the number of gametocytes present was not so important as their 'quality'. He found that the male gametocytes from patients who were 'good infectors' exflagellated within 15 min. in a saturated atmosphere at 25° C. Boyd (1942), however, did not find exflagellation to be a reliable criterion of the power of the gametocytes to infect mosquitoes; gametocytes of *P. vivax* from some patients produced poor infections in mosquitoes even though their density was high and exflagellation of the male gametocytes frequent. In some experiments with the same species of parasite Knowles & Basu (1943) obtained negative results when *Anopheles stephensi* was fed on patients with very heavy gametocyte counts. Muirhead-Thomson (1954*b*) described subjects whose blood was non-infective to *A. gambiae* although it contained an abundance of gametocytes, the highest proportion of these non-infective carriers occurring amongst infants. Similar observations have been made upon *Plasmodium gallinaceum* by the writer, a poor rate of infection as determined by the oocyst count, or complete absence of infection, being observed in some batches of mosquitoes fed upon birds whose blood contained large numbers of gametocytes of which the males exflagellated well. These experiments were all made during the winter months.

Boyd (1949) has summarized the factors which determine the infectivity of gametocytes to mosquitoes as their numerical density, their maturity as demonstrated by exflagellation, parity or disparity in the proportion of the sexes, and an elusive, as yet undefined characteristic which James called 'quality'.

There is evidence that, in *P. gallinaceum*, the stage of the infection influences the infectivity of the gametocytes to *Aëdes aegypti*. Lumsden & Bertram (1940) found that the maximum number of oocysts was usually attained when the mosquitoes were fed on birds before the total parasite count had reached its peak. Cantrell & Jordan (1946) confirmed this, the highest oocyst counts in mosquitoes fed upon birds infected by blood-inoculation occurring on the day prior to that of the peak of parasitaemia; thereafter, they declined rapidly. It was suggested that some substance required for gametocyte survival and development might become exhausted at the peak of parasitaemia. To test this hypothesis normal blood was transfused into highly parasitized birds; in two out of three experiments there was an enhancement of the infectivity of the gametocytes, although the proportion of parasitized red cells was substantially reduced.

In a series of experiments in which *A. aegypti* were fed through membranes upon chicken blood infected with *Plasmodium gallinaceum* from blood-inoculated infections, Eyles (1952) attempted to discover the factors adversely affecting the infectivity of the gametocytes. In infected blood withdrawn at the peak, or immediately after the peak of parasitaemia, the substitution of serum from a normal bird for that of the infected blood frequently enhanced the infections produced in mosquitoes as compared with those produced by the unaltered infected blood. The addition of washed erythrocytes from a uninfected bird also led to an enhancement of infection in the mosquitoes in most experiments. Conversely, the mean oocyst counts were reduced in mosquitoes fed upon parasitized blood taken 2 days before the peak of parasitaemia if serum from heavily infected chickens at the peak of infection was added. Eyles concluded that factors resulting from the physiological condition of the vertebrate host can influence the intensity of infection in the mosquito.

Until recently, it was believed that, in human malaria, blood with a low gametocyte density was not infective to mosquitoes, though the numbers of gametocytes present might be sufficient for detection in blood films without too exhaustive a search. Green (1929), working in Malaya, estimated that the minimum number of gametocytes of *P. falciparum* necessary to infect *Anopheles maculatus* was 42 per cu.mm. of blood or 1 per 200 leucocytes, whereas 10 gametocytes of *Plasmodium vivax* per cu.mm. or 1 per 1000 leucocytes, and 27 gametocytes of *P. malariae* per cu.mm. or 1 per 300 leucocytes, were sufficient to produce an infection in this species of mosquito. In *Anopheles elutus*, however, Kligler & Mer (1937), in some instances obtained a relatively high percentage of infection with *Plasmodium falciparum* when the blood contained only 5 gametocytes per cu.mm. More recently, Young, Hardman, Burgess, Frohne & Sabrosky (1948) have shown that infections with this parasite can be obtained when the density of gametocytes is even lower; indeed some batches of *Anopheles quadrimaculatus* became infected when fed upon negroes in whom the crescents were too few to be detected, though the proportion of mos-

quitoes infected was small. The infections produced in mosquitoes fed upon subjects with low gametocyte densities were light, few showing as many as 10 oocysts per stomach. In Jamaica, Muirhead-Thomson & Mercier (1952) have obtained infections in 12 out of 37 mosquitoes (*Anopheles albimanus*) fed upon a 3 year-old negro child in whom no crescents could be found, and in 12% of another batch fed upon a subject in whom crescents were fewer than 1 per cu.mm. In West Africa infections were obtained in *A. gambiae* when the gametocyte threshold was below 0·3 per 100 leucocytes, though the number of oocysts produced in each infection was small (Robertson, 1945). Muirhead-Thomson (1954*a*, *b*), working on the West Coast of Africa, also has obtained infections of *Plasmodium falciparum* in *Anopheles gambiae* when the density of crescents in the blood was very low. The proportion of mosquitoes infected from these cryptic carriers was usually 10% or less, and the intensity of infection was only 1–3 oocysts per stomach. In striking contrast were individuals whose blood was non-infective to mosquitoes, although crescents were abundant. Muirhead-Thomson (1954*b*) concludes that in the routine examination of a community for malarial infection, the presence or apparent absence of crescents in the blood may give a misleading idea of the importance of individuals as a source of infection to mosquitoes.

It is obvious from these observations made upon a variety of species of *Plasmodium*, that gametocyte density is not necessarily a reliable indication of infectivity to susceptible mosquitoes.

IX. THE EFFECT OF ANTIMALARIAL DRUGS UPON GAMETOCYTES

Antimalarial compounds have been found to differ in the intensity of their action upon different stages of the life cycle. This is not surprising since different stages of the cycle take place in different tissues of the host, and whereas the gametocytes merely increase in size, the asexual parasites not only increase in size but their nuclei divide frequently,.

Soon after its discovery, pamaquin was found to have a more marked effect upon the gametocytes, particularly those of *P. falciparum* (Mühlens, 1926; Roehl, 1927), than upon asexual stages. Whereas quinine, in therapeutic doses, had no effect upon the infectivity of crescents to *Anopheles albimanus*, Barber, Komp & Newman (1929) found that a single dose of pamaquin as small as 0·2 mg. per kg. of body weight prevented infection of the mosquitoes, not only on the day of dosage, but for several succeeding days. Although they were non-infective, gametocytes continued to increase in number in the blood of the patient after administration of the drug, and exflagellation of the male gametocytes occurred readily. More recently, Mudrow-Reichenow (1953) has shown that pamaquin and the related 8-aminoquinoline compound primaquine, when administered in small doses to chickens infected with *Plasmodium gallinaceum*, inhibited the development of the gametocytes in *Aëdes aegypti*. These compounds were found to inhibit exflagellation of the male gametocyte of *Plasmodium cathemerium*. It is clear, however, that the non-infectivity of the gametocytes to mosquitoes was not due solely to the inhibition of exflagellation by the drug, since exflagellation was observed by Barber

and his colleagues in the microgametocytes from carriers of *P. falciparum* treated with the drug though their blood proved to be non-infective to mosquitoes. Similar observations were made by Mackerras & Ercole (1949*a*, *b*), who found that a dose of 10 mg. of pamaquin was sufficient to render the blood of a crescent carrier non-infective to mosquitoes within 15 hr., though the male gametocytes were still capable of exflagellation.

In birds infected with *P. lophurae* and treated with quinine, the gametocytes were affected more slowly than the asexual parasites, and in those treated with mepacrine the gametocytes and the larger schizonts were the last to undergo a change (Hewitt & Richardson, 1943). In *P. vivax* Mackerras & Ercole (1949*a*) found that quinine did not affect mature gametocytes though it affected the growth of the young forms and that of the trophozoites, and mepacrine, in low concentrations but sufficient to destroy the majority of the trophozoites, was without effect upon the developing gametocytes.

The action of proguanil against *P. falciparum* was found by Black (1946) to be directed against the schizogonic stages, and in *P. vivax* its lethal action was exerted only upon the dividing nucleus (Mackerras & Ercole, 1948). Similar observations have been made upon *P. berghei* (Thurston, 1951) and *P. cynomolgi* (McFadzean, 1951). It was observed that whereas proguanil cleared the asexual parasites of *P. falciparum* from the patients' blood, the crescents appeared to be unaffected by its action (Maegraith, Adams, King, Townshend, Davey & Havard, 1945). These observations were confirmed by Fairley (1946) who found that the drug, when given in therapeutic doses, had no effect upon either the numbers or appearance of the gametocytes of *P. vivax* or *P. falciparum*.

Fairley (1946) observed that when mosquitoes were fed upon patients who had received small doses of proguanil, although exflagellation and fertilization might take place, the oocysts failed to complete their development on the stomach wall. This inhibitory effect of the drug was found to persist until the tenth day following a course of 300 mg. of proguanil daily for 10 days; oocysts and sporozoites developed normally in mosquitoes fed upon a patient on the twelfth day, or thereafter, following the end of drug treatment (Mackerras & Ercole, 1948). Amongst mosquitoes fed upon patients who had received 1-day treatment with proguanil, the percentage infected declined over the first 3 days following treatment, but rose steeply in mosquitoes fed on the fourth day; mosquitoes fed on the eighth day were the first to show complete development of sporozoites in the salivary glands (Shute & Maryon, 1948). Thus, unlike pamaquin, proguanil does not prevent infection of the mosquito, but very small quantities of the drug imbibed with the infected blood affect the vitality of the parasites so that they are unable to complete their development in the insects' tissues.

An increase in the numbers of gametocytes produced on the second and third day of treatment with proguanil, which was greater than that produced during treatment with quinine, mepacrine, or pamaquin, was observed by Mackerras & Ercole (1948) in patients infected with *P. vivax*. In *P. falciparum* Shute & Maryon (1951) found than an increase in gametocyte production occurred in cases in which the primary attack had been aborted by treatment with antimalarial drugs, and

they were of the opinion that inadequate treatment of the primary attack, irrespective of the drugs used, favoured gametocyte production. In twelve cases treated with proguanil gametocytes were numerous during recrudescence. When sulphamethazine, which has a very weak schizonticidal action, was given over a period of 24 hr., gametocytes appeared in enormous numbers some 10 or 14 days later. Findlay, Maegraith, Markson & Holden (1946) previously had observed showers of gametocytes in the blood of patients infected with *P. falciparum* both during and after treatment with this drug. In a Panama strain of this species Ramakrishnan, Young, Jeffery, Burgess & McLendon (1952) observed a high density of gametocytes in patients treated with proguanil, but they were of the opinion that more data were needed before it could be assumed that the drug actually produced an increase in the number of gametocytes.

Srivastava, Chakrabarti & Mukherjee (1953) observed that although in *P. vivax* infections treated with pyrimethamine the clearance of gametocytes was as rapid as of the asexual forms, in *P. falciparum* infections the crescents, in most cases, persisted irrespective of the regimen of treatment with the drug, and, in some cases, continued to increase in number until a stage of heavy parasitaemia was reached although asexual parasites might be absent.

Since proguanil, sulphadiazine, 2:4-diaminopteridines and pyrimethamine all act upon the early stages of the developing schizont (Thurston, 1953), in which there is a rapid synthesis of nuclear material, it is not surprising that they are less effective upon the gametocytes. There are, however, indications that sulphadiazine proguanil and 2:4-diamino-6:7-di*iso*propylpteridine may lead to an increase in the production of gametocytes. It was observed by Bishop & Birkett (1948) that, in the majority of chicks infected with proguanil-resistant strains of *P. gallinaceum*, the proportion of gametocytes to asexual parasites was greater than in the normal strain, though both strains were maintained in the same way. When a proguanil-resistant strain which had not yet become fully resistant to the drug was exposed to a slightly increased dose of drug, the asexual parasites, particularly the mature schizonts, were reduced in number and abnormal in appearance, whereas the gametocytes were numerous. More recently, Bishop (1954) has subjected strains of *P. gallinaceum*, maintained in young chicks by intravenous injection of infected blood, to small but effective doses of sulphadiazine, proguanil or 2:4-diamino-6:7-di*iso*propylpteridine. In these strains, in which resistance to the drug was slowly developing, the numbers of gametocytes, though variable, were much greater than in the parent strain maintained in a similar manner but not exposed to the action of the drug. The greatest number of gametocytes was produced in the sulphadiazine-treated strain. Since the numbers of gametocytes present in untreated birds inoculated with the drug-treated strains, as well as in birds receiving the drug, were high, it was concluded that the increase in number of gametocytes was not due to the immediate and direct effect of the drug upon the parasites or upon the host; this deduction was supported by the observation that no change in gametocyte number occurred when parasites were treated with sulphadiazine or proguanil for a few days only. The effect of the drugs upon gametocyte-production appeared to be a long-term one, and seems to be limited to drugs related to *p*AB-folic acid

metabolism, since no increase in gametocyte number was observed in strains of *P. gallinaceum* treated with pamaquin or metachloridine, though the pamaquin-treated strain developed a low-grade resistance to pamaquin, and the metachloridine-treated strain became highly resistant to metachloridine; nor was an increase in gametocyte number observed in strains treated for long periods with mepacrine or chloroquine.

X. SUMMARY

Although many interesting observations have been made upon gametogenesis in Haemosporidiidea many fundamental problems await solution. Our knowledge of the nuclear changes occurring during the development of the gametes and the fertilization of the female gamete is still incomplete. Little is known of the factors which control gametocyte production, though there is evidence that in some species, i.e. *Leucocytozoon simondi* and *Haemoproteus columbae* in the duck, it is influenced by the physiological stage of the host. The type of host and the method of transmission have also been shown to affect the numbers of gametocytes formed in some species. No correlation has been found in *Plasmodium*, the genus in which this subject has been studied extensively, between acquired immunity and gametocyte formation. Although a broad relationship has been demonstrated in *Plasmodium* between the density of gametocytes in the blood and the oocyst rate and density assessed in susceptible mosquitoes, hosts heavily infected with apparently ripe gametocytes may prove non-infective to susceptible mosquitoes. In birds heavily infected with *P. gallinaceum*, serum taken at the peak of parasitaemia has been shown to affect adversely the infectivity of gametocytes to mosquitoes. Pamaquin and primaquine, in small doses, inhibit the development of gametocytes completely, whereas proguanil may not prevent the formation of oocysts though it inhibits their subsequent development. High densities of gametocytes have been observed in patients treated with proguanil or sulphamethazine. An actual increase in the number of gametocytes produced, as compared with the untreated control strain, was observed in strains of *P. gallinaceum* in process of developing resistance to sulphadiazine, proguanil or 2:4-diamino-6:7-di*iso*propylpteridine as a result of treatment with those drugs.

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OCCURRENCE OF TWO SPECIES OF *CREPIDOSTOMUM* IN BROWN TROUT (*SALMO TRUTTA* L.) FROM NORTH-EAST IRELAND, WITH SPECIAL REFERENCE TO *CREPIDOSTOMUM METOECUS* BRAUN 1900

By M. PAULINE CORBETT

Department of Zoology, The Queen's University of Belfast

During the course of preliminary studies on the helminth fauna of local fresh-water fishes two species of *Crepidostomum*, obtained from the intestine of brown trout, were identified as *C. farionis* (Müller) and *C. metoecus* Braun. Although *C. farionis* is a common parasite of the fresh-water salmonoid fishes of the Northern Hemisphere, in Ireland it has been recorded only from trout (*Salmo trutta*). The first Irish specimens were recorded by Southern (1912) as *Stephanophiala laureata* (Zeder), and came from fish from a stream in Clare Island and from the Owenwee River, Westport. Later, Vickers (1951) found that trout from the River Moyola, Co. Londonderry, and River Ravarnett, Co. Down, were infected with this species. Prior to the present study, *C. farionis* was the only representative of the genus to have been recorded from the British Isles. *C. metoecus* is therefore a new record, not only from Ireland, but from the British Isles as well. Elsewhere it is known to occur in Sweden [=*C. suecicum* Nybelin, 1933], Austria, and possibly also in Thuringia, where cercariae were found which Nöller (1928) assigned to *C. metoecus*.

C. metoecus was first described by Braun (1900*a*) as *Distomum metoecus*. Later (1900*b*) he supplemented his original description and used the species as the type upon which he set up the genus *Crepidostomum*. He obtained his specimens at the Vienna Museum, where they were labelled as having been taken from two species of noctules. Odhner (1905, 1910) also used the Vienna Museum material when he amended some of Braun's work and discussed the systematic position and relationships of the genus *Crepidostomum*. More recently this genus was reviewed by Hopkins (1934) in a monograph on the 'papillose Allocreadiidae'. Hopkins examined most of the American and European species of *Crepidostomum*, and using these, together with the relevant literature, revised the generic description. The material he worked on included Braun's original specimens of *C. metoecus*. He considered these to be identical with *C. suecicum* obtained from various fresh-water fish in Sweden. He examined material lent by the University of Berlin Museum and recorded *C. farionis* and *C. metoecus* as occurring in *Perca fluviatilis* from Vienna. It is interesting to note that these two species have also been observed by the present writer in the intestine of the same individual (*Salmo trutta*).

With only the material in the Vienna Museum to substantiate it, *C. metoecus* is always included among the parasites of bats. This material was labelled as coming from two species, *Nyctalus maximus* (Fatio) and *N. noctula* (Schreber). Regarding the locality of these noctules Hopkins says: 'Braun does not state where the specimens in the Vienna Museum were collected, but both these bats are Central

European species according to Brohmer (1929) in *Die Tierwelt Mitteleuropas.*' But whereas *N. maximus* is confined to central and south-eastern Europe, *N. noctula* has a very wide distribution. Harrison Matthews (1952) says that the latter is known to inhabit all temperate Europe and Asia and that it is common throughout Wales and most of England. In view of this, and the number of helminths which have been recorded from this bat, it seems remarkable that the occurrence of *C. metoecus* in bats has never been confirmed.

The anomaly that two such widely different hosts as a fish and a bat, should harbour the same genus of parasite, was noted by Braun who explained that the larval stages of both *C. metoecus* and *C. farionis* might be passed through insect larvae forming food common to both hosts. Hopkins suggests that fish are the 'natural hosts' and that bats are only 'occasional' or 'accidental' hosts. Flukes are known to require a specific environment for their development, especially within their definitive host, and no attempt has been made to explain how a form normally parasitic in a cold-blooded animal adapts itself to a very different environment, including a temperature of 98° F.

Specimens were first obtained by me from trout from the Upper Bann, close to Banbridge in May 1953. Each of the four trout examined was found to be parasitized. Thirty-six specimens were recovered, twenty-seven of which were *C. metoecus* and the remainder *C. farionis*. In April 1954, four trout from the Silent Valley Reservoir, Co. Down, were examined. Again, each was found to be infected with *C. metoecus*, seventy-three specimens of which were recovered. In addition, two of these trout contained four and five specimens respectively of *C. farionis*. Three trout taken from the River Bush at Stranocum, Co. Antrim, during July 1954 were found to be free from *C. farionis*, but two of these fish were infected with a few small specimens of *C. metoecus*, located in the pyloric region of the stomach and upper intestine.

In the present study, 70% alcohol was used as a fixative and whole mounts stained in Gower's carmine and Ehrlich's haematoxylin.

It should be pointed out that there are some minor differences between the specimens of *C. metoecus* so far recovered from Irish trout and those previously described by other workers. The former are a little broader proportionately. In addition, they tend to contain a greater number of intra-uterine eggs (13 to 35 as opposed to 1 to 24), and these eggs are slightly larger (57–79 μ long, 30–47 μ wide as opposed to 53–66 μ long, 30–47 μ wide.)

The gut of the large lake trout, or 'dollachan', of Lough Neagh has been examined periodically and has been found to be completely free from trematode infection. Since the Upper Bann flows into Lough Neagh, this is contrary to expectation. The absence of *Crepidostomum* from the dollachan may be due to overcrowding by *Eubothrium crassum* (Bloch) which is invariably present in large numbers.

My thanks are due to Messrs Vickers and Deane and Professor Gresson for providing me with the trout from which *C. farionis* and *C. metoecus* were obtained.

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THE COMPLEMENT-FIXATION TEST IN BILHARZIASIS

II. PREPARATION AND PRESERVATION OF ANTIGENS FROM *SCHISTOSOMA MANSONI* WORMS EXTRACTED IN COCA'S SOLUTION

By M. ELIAKIM and A. MICHAEL DAVIES*†

The Medical Research Laboratories, Medical Corps, Israel Defence Forces and the Israeli Institute for Biological Research, Ness Ziona, P.O.B. 19, Israel

The efficiency of different extracts of adult *Schistosoma mansoni* worms in the complement-fixation test (C.F.T.) for bilharziasis has been previously examined (Eliakim & Davies, 1954), and an extract in Coca's solution was found to be the most specific. Because of the paucity of the material it is necessary to elaborate a method for thorough extraction of the worms, and since a disadvantage of the 'Coca extract' is its relative instability (Bozicevich & Hoyem, 1947), it is also of importance to seek a way of preserving it for long periods of time.

This paper reports the results of experiments in this field.

MATERIAL AND METHODS

Worms were collected from infected mice as reported previously (Davies & Eliakim, 1954). Samples collected over a period of 4 months, were kept dry in the refrigerator, then lyophilized in lots of 20, 50, and 100 mg.

A. *Extractions*

Two or three extractions were performed on each of six aliquots of 20 mg. worms from freshly opened ampoules. All extractions were made in rubber-corked tubes, left to stand in a slanting position, and were so arranged as to complete a series on the same day. The first extraction was performed by suspending 20 mg. worms in 2 ml. Coca's solution (1:100), except where otherwise indicated, and using one of the methods described below. Extracts were centrifuged at 3000 r.p.m. for 20 min., and the supernatants decanted for simultaneous titration on the following day. The tubes were weighed together with the worms before the extraction, and, after thorough drying in a vacuum desiccator at 2° C., subsequent to extraction. Second and third extractions were performed in the same volume of fluid as the first, but not necessarily by the same method. Each extract was also titrated at the same time as the subsequent extracts from the same sample. The maximal time needed for three extractions of one worm sample was 8 days and no drop in titre of the first extract occurred in this period. The methods were as follows:

I. At 37° C., in the incubator for 1 hr., with vigorous shaking at 10 min. intervals, then left to stand at 28° C. for 23 hr.

* Present Address: Department of Hygiene of the Medical School of the Hebrew University and Hadassah, Jerusalem, Israel.

† This work was carried out in the Department of Epidemiology, Israeli Institute for Biological Research, Ness Ziona.

II. At 2° C., in the refrigerator, for 6 days, with daily shaking.

III. At 56° C., in the water-bath, for 1 hr. with vigorous shaking at 10 min. intervals.

IV. At 100° C., in the water-bath, for 15 min.

V. At 56° C., in the water-bath, for 4 hr., with vigorous shaking at 30 min. intervals.

VI. As in method I, except that the worms were suspended in 10 ml. Coca's solution (1:500).

B. *Preservation*

Two batches of 100 mg. worms were extracted according to method I; after centrifugation, the supernatants were decanted, pooled, and sealed in aliquots of 0·3 ml. Four groups of eight samples were preserved for serial titrations in the following ways:

(*a*) At 2° C.

(*b*) At 2° C., after the addition of cysteine to a final concentration of 0·1 %.

(*c*) At −20° C.

(*d*) Lyophilized.

C. *Antigen titration*

Each antigen was titrated against a rising amount of complement in the presence of a constant quantity of pooled 'positive' serum from six patients with bilharziasis (diluted 1:4). For the purpose a large pool of serum was prepared, divided in 2 ml. samples, and kept in sealed bottles at −20° C., with 1 : 10,000 sodium merthiolate as preservative. It was inactivated at 56° C. for 30 min. before use. Complement titre, estimated in the presence of the respective antigen (diluted 1 : 10) and pooled serum from six healthy subjects (diluted 1 : 4), was taken to be the last tube showing full haemolysis.* Comparative titrations were performed simultaneously and with the same pooled positive serum. Results were graded as ± = less than 25 % inhibition of haemolysis, + = 25–50 %; + + = 50–75 %; + + + = 75–100 %; and + + + + = 100 %.

D. *Complement-fixation test*

This was performed by the method previously described (Eliakim & Davies, 1954). The sera of twenty-five non-treated patients with chronic bilharziasis and twenty-five healthy subjects, recent immigrants from Europe, were tested.

RESULTS

A. *Extraction of adult* Schistosoma mansoni *worms at different temperatures*

Table 1 shows the results of one of three similar experiments. The titration of the first extracts according to methods I–III gave similar results, although less material was extracted by method III. In the other two experiments, extract II was less potent than I and III. Boiling the worms for 15 min. (IV) yielded an

* The titre of the complement was not affected by the presence of antigen extracted or preserved by different methods.

Table 1. *Titration of antigens t*

First extraction method ...	I Complement (M.H.D.)			II Complement (M.H.D.)			III Complement (M.	
Antigen dilution	2	3	5	2	3	5	2	3
1 : 250	++++	++++	++++	++++	++++	++++	++++	++++
1 : 500	++++	++++	+++	++++	+++	−	++++	+++
1 : 1000	+++	−	−	++	−	−	+++	±
1 : 1500	±	−	−	±	−	−	±	−
1 : 2000	−	−	−	−	−	−	−	−
1 : 3500	−	−	−	−	−	−	−	−
mg. extracted from 20 mg. worms ...		5·2			5·2			3·0
Second extraction method ...		I			I			I
1 : 250	++++	++++	++++	++++	++++	++++	++++	++++
1 : 500	++++	++++	++++	++++	++++	++++	++++	++++
1 : 1000	++++	+++	±	++++	++++	+	++++	++++
1 : 1500	+++	++	−	++++	++	−	++++	++
1 : 2000	++	±	−	++++	+	−	++	−
1 : 2500	+	−	−	++	−	−	±	−
mg. extracted from residue ...		4·8			4·8			4·6
Third extraction method ...		I			I			
1 : 250	++++	++++	++++	++++	++++	++++		
1 : 500	++++	+++	±	++++	+++	±		
1 : 1000	++	−	−	++	−	−		
1 : 1500	+	−	−	+	−	−		
mg. extracted from residue ...		2·4			1·2			
Total extracted from 20 mg. worms ...		12·4			11·2			7·6

Table 1. *Titration of antigens titrated at different temperatures*

(M.H.D.)	III Complement (M.H.D.)			IV Complement (M.H.D.)			
5	2	3	5	2	3	5	
+ ++++	++++	++++	++++	++++	+	±	+
−	++++	+++	+	+++	±	−	+
−	+++	±	−	−	−	−	
−	±	−	−	−	−	−	
−	−	−	−	−	−	−	
−	−	−	−	−	−	−	
	3·0			2·4			
	I			I			
+ ++++	++++	++++	++++	++++	++++	+++	+
+ ++++	++++	++++	+++	++++	++	−	+
+ +	++++	++++	+	++++	+	−	
−	++++	++	−	++	±	−	
−	++	−	−	−	−	−	
−	±	−	−	−	−	−	
	4·6			4·7			
+ ++++							
±							
−							
−							
	7·6			7·1			

Key

I. 37° C./1 hr. and 28° C./23 hr. at 1 : 100.
II. 2° C./6 days.
III. 56° C./1 hr.
IV. 100° C./15 min.
V. 56° C./4 hr.
VI. 37° C./1 hr and 28° C./23 hr. at 1 : 500.

ures

	V Complement (M.H.D.)			VI Complement (M.H.D.)		
H.D.)	2	3	5	2	3	5
5	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
±	+ + + +	+ + +	+ +	+ + + +	+ + + +	+ + + +
−	+ +	−	−	+ + + +	+ + + +	+ + + +
−	±	−	−	+ + + +	+ + +	+ +
−	−	−	−	+ + +	+ +	±
−	−	−	−	+ +	−	−
	4·8			7·2		

	V			VI		
+ + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
−	+ + + +	+ + + +	−	+ + + +	+ + + +	+ + + +
−	+ +	−	−	+ + + +	+ + +	+
−	+	−	−	+ + +	+ +	−
−	−	−	−	+ + +	+	−
−	−	−	−	+ +	−	−
	3·2			2·0		

r. at 1 : 100.

VI		
+ + + +	+ + + +	+ +
+ + + +	+ + + +	−
−	−	−
−	−	−
	0·4	

. at 1 : 500.

8·0 9·6

extract of lower potency, but subsequent extraction of the worm residue, by method I, proved that the active principle had not been destroyed by the heat. The most potent extract was obtained by method VI in which the largest quantity of material was also extracted.

The second extraction gave equally or (usually) more potent antigens than the first (except when the latter was performed by method VI) in spite of the fact that, in several cases, less material was extracted (see Table). Antigen was also found in the third extractions when these were performed. The results of all three experiments were very similar.

If the antigen content in the extracts is estimated in units according to the formula

$$\text{A.U.} = \frac{\text{titre}^*}{\text{mg. worms extracted} \times 100}$$

it is evident that the concentration of the active principle increased with subsequent extractions (Table 2).

B. *Complement-fixation test with antigens obtained by repeated extractions of worms*

The specifity of the extracts obtained by methods I and VI was tested. It is seen from Table 3 that the first extracts obtained by both methods gave positive results with the sera of twenty-one of twenty-five patients. The second extract by method I gave equivalent results, while the second extract by method VI and the third extracts by both methods were less specific. The difference was also evident in the titre of the sera which was lower when performed with the latter extracts.

The sera of the control subjects gave negative results with all extracts.

C. *Preservation of the extract of adult* Schistosoma mansoni *worms in Coca's solution by different methods*

Table 4 shows the results of comparative titrations of antigens preserved as indicated, at approximately monthly intervals. The titre of the antigen pool, immediately after extracting the worms, was equal to that of the antigen preserved at 2° C., 30 days later. It is evident that the titre remained unchanged for 1 month when the extract was preserved by methods *a* and *b*, thereafter dropping gradually. The drop in titre was more pronounced when the antigen was kept frozen or lyophilized and was evident within the first month. The frozen samples sometimes showed a brownish precipitate when thawed. The addition of cysteine to the extract had no effect on the titre.

D. *Preservation of adult* Schistosoma mansoni *worms as dry powder*

The titre of antigen obtained by extraction of lyophilized worms from freshly opened ampoules remained constant for $3\frac{1}{2}$ months but showed a slight drop, probably without significance, 5 and 6 months after lyophilization.†

* Maximal dilution of antigen showing 50 % or more inhibition of haemolysis with 2 M.H.D. complement.

† A sample of lyophilized *S. mansoni* worms received through the courtesy of Lt.-Col. A. S. Benenson, from the Tropical Research Medical Laboratory, A.P.O., N.Y., showed a high titre 18 months after its original preparation.

In a previous batch of worm powder kept at 2° C., with no special precaution to avoid rehydration, the titre of complement-fixing antigen dropped markedly in 40 days. However, another sample, kept under the same conditions but thoroughly dry showed exactly the same behaviour as the lyophilized controls for a period of 4 months.

Table 2. *Antigen activity in the different extracts expressed in units*

First extraction method	...	I	II	III	IV	V	VI
Units		1·9	1·9	3·3	2·1	2·1	4·9
Second extraction method	...	I	I	I	I	V	VI
Units		4·2	5·2	4·3	3·2	3·1	12·0
Third extraction method	...	I	I	—	—	—	VI
Units		4·2	8·3	—	—	—	12·5

Table 3. *Results obtained with antigens from repeated extractions of worms*

Method of extraction ...	I						VI					
Extract ...	1		2		3		1		2		3	
	+	−	+	−	+	−	+	−	+	−	+	−
Patients	21	4	21	4	14	6	21	4	19	6	16	9
Controls	0	25	0	25	—	—	0	25	0	25	0	15
	Titre = titre > titre						Titre > titre > titre					

DISCUSSION

It has been suggested that the antigen in the C.F.T. for bilharziasis is lipoprotein in nature (Eliakim & Davies, 1954). Its low solubility in Coca's solution, demonstrated by the presence of active principle in three successive extractions of one worm sample, conforms to this view. The solubility was not markedly increased by changing the period of extraction or the temperature at which it was performed but it rose significantly when the quantity of the extracting fluid was increased. It seems that the first extraction removed most of the inert material from the worms as in the subsequent ones the antigen was obtained in higher concentration.

The use of either two extractions of one worm sample by method I or, if more potent material is needed, one extract by method VI, is recommended for routine examinations.

The extract of adult *S. mansoni* worms (method I) retains a suitable titre for 1 month when kept in the refrigerator. Freezing and, less so, lyophilization rapidly destroy its activity. Minning (1941) attributed the rapid drop in titre of saline extracts of adult worms to the phenol, which was added as preservative, but Bozicevich & Hoyem (1947) reported that ageing had a marked deleterious effect on saline extracts devoid of phenol.

Whatever the cause of the drop in titre may be it seems that, so far, the best method to preserve the antigen for longer periods of time is in the form of worm powder lyophilized in small samples, or kept in the refrigerator thoroughly desiccated; fresh extracts should be prepared whenever needed for routine work.

Table 4. *Serial titrations of an extract of adult* Schistosoma mansoni *worms in Coca's solution, preserved by different methods*

Method of preservation	2° C.			2° C. with 0·1 % cysteine			−20° C.			Lyophilized		
Complement (M.H.D.)	2	3	5	2	3	5	2	3	5	2	3	5
Dilution of antigen						Titration after 30 days						
1 : 250	++++	++++	++++	++++	++++	++++	++++	++++	+++	++++	++++	+++
1 : 500	++++	++++	++++	++++	++++	++++	++++	+++	−	++++	++++	±
1 : 1000	++++	++++	+	++++	++++	−	+	−	−	+++	+	−
1 : 1500	++++	++++	−	++++	+++	−	−	−	−	−	±	−
1 : 2000	+++	+	−	+	−	−	−	−	−	−	−	−
						Titration after 60 days						
1 : 250	++++	++++	±	++++	++++	++	+++	++	−	++++	+++	−
1 : 500	++++	+++	−	++++	++++	−	±	−	−	++++	++	−
1 : 1000	++	−	−	+++	−	−	−	−	−	+++	−	−
1 : 1500	+	−	−	±	−	−	−	−	−	−	−	−
						Titration after 90 days						
1 : 250	++++	++++	±	++++	++++	++	+++	+	−	++++	+++	−
1 : 500	++++	+++	−	++++	+	−	+	−	−	++++	+++	−
1 : 1000	++++	−	−	++	−	−	−	−	−	++++	±	−
1 : 1500	++	−	−	±	−	−	−	−	−	−	−	−
						Titration after 125 days						
1 : 250	++++	++++	−	++++	++++	+	++	+	−	++++	++++	−
1 : 500	+++	++	−	++++	++	−	+	−	−	+++	+++	−
1 : 1000	+	−	−	+	−	−	−	−	−	+++	+	−
1 : 1500	−	−	−	−	−	−	−	−	−	−	−	−
						Titration after 170 days						
1 : 250	++++	++++	−	++++	++++	−	++	+	−	+++	++	−
1 : 500	+++	++	−	+++	++	−	+	−	−	++	−	−
1 : 1000	+	−	−	+	−	−	−	−	−	+	−	−

SUMMARY

1. Adult *S. mansoni* worms were extracted in Coca's solution at various temperatures by different methods. The most potent antigen was obtained by extracting a worm suspension, diluted 1 : 500, at 37° C. for 1 hr., with vigorous shaking at 10 min. intervals, followed by 23 hr. at 28° C. Second extraction of worm samples gave highly active material and third extractions also contained antigen.

2. Complement-fixation tests, performed with the sera of twenty-five patients with chronic bilharziasis and twenty-five healthy subjects showed that the most specific results were obtained with either the first two extracts of worms, suspended in 100 parts of Coca's solution, and extracted for 1 hr. at 37° C. and 23 hr. at 28° C., or with the first extract of worms, suspended in 500 parts and extracted in the same manner.

3. Samples of an extract in Coca's solution were preserved for 170 days by different methods, and their activity was tested at approximately monthly intervals. The antigen, with or without the addition of 0·1 % cysteine, retained its titre for 1 month, when kept in the refrigerator. Freezing, or lyophilization of the extract caused a rapid drop in titre.

4. Titration of extracts of lyophilized worms, from freshly opened ampoules, showed that a constant titre was retained for 3½ months followed by a slight drop after 5–6 months. The worm powder retained its antigenic potency when kept, thoroughly desiccated, in the refrigerator for 4 months, but lost it in 40 days when partial rehydration occurred.

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ON THE GENUS *EUCAMPSIPODA* KOL. AND *DIPSELIOPODA* n.g. (NYCTERIBIIDAE, DIPTERA)

By O. THEODOR

Department of Parasitology, The Hebrew University, Jerusalem

(With 53 Figures in the Text)

The genus *Eucampsipoda* was created by Kolenati in 1857 for *Eucampsipoda hyrtlii*, the common parasite of *Rousettus aegyptiacus*, and *Nycteribia fitzingeri* Kol., 1856, which he later considered as synonym with *N. aegyptia* Macquart, 1851. In his paper of 1863 Kolenati gives illustrations of *E. hyrtlii*, but not of *E. aegyptia* as he had in the meantime lost his specimens. Macquart's description does not permit the recognition of the species, and it is even doubtful whether he was dealing with an *Eucampsipoda* at all. Speiser (1901) adds only a few details to Kolenati's description of *E. hyrtlii*, but introduces an unfortunate complication by incorrectly identifying specimens from Burma and Sumatra as *E. hyrtlii*. Later (1908) Speiser identified specimens from the Comoro Islands as *E. hyrtlii*, but this record has also become doubtful, as will be shown later. Scott (1914) accepts Speiser's identification of the Indonesian material as *E. hyrtlii*, and describes and figures a female specimen from Ceylon as *E. hyrtlii*, which is in fact *E. latisternum* Schuurm. Stekh. Scott (1925) identified specimens from South Africa as *E. hyrtlii* which on re-examination proved to belong to a new species. He also mentions Madagascar as a locality for *E. hyrtlii* without giving details. The distribution of *E. hyrtlii* was thus apparently extremely wide, throughout Africa, from the Cape Province to Egypt and beyond into Palestine, Comoro Islands, Madagascar, Ceylon, Burma and Sumatra.

In 1924 Ferris described *E. philippinensis*, and in 1939 Schuurmans Stekhoven described *E. latisternum* from Java.

In connexion with a study of the palaearctic Nycteribiidae and in an attempt to define their distribution, some of the material on which the above records of *E. hyrtlii* were based was re-examined. It appeared that the South African material belongs to a new species which is widely distributed throughout Africa, while the Oriental material belongs either to *E. latisternum* or to several other species which will be described as new in this paper. Material from Madagascar also proved to belong to a new species. Speiser's record from the Comoro Islands thus becomes doubtful and his material will have to be re-examined.

I have to thank the authorities of the British Museum (Nat. Hist.), the Chicago Natural History Museum, the Zoological Museum, Genoa, the Zoological Museum of the University, Berlin, and the Naturhistorische Museum, Wien, for putting their material at my disposal.

Genus **Eucampsipoda** Kolenati, 1857

Type species: *Eucampsipoda hyrtlii* Kolenati, 1856.

The genus is one of the best defined of the family. Together with the genus *Cyclopodia* and the new genus *Dipseliopoda*, described below, it forms a subdivision of the family which is characterized by the structure of the thorax, the reduced segmentation of the abdomen of the female and the structure of the genitalia.

All species of *Eucampsipoda* resemble each other closely in the structure of head and thorax, and in the segmentation and chaetotaxy of the abdomen, which explains why so many species were considered as *E. hyrtlii* for a long time. Nearly the only chaetotactic characters of systematic value were found to be in the arrangement of the long setae on the dorsum of the abdomen of the female, and only in a few species such characters exist in the male. Differentiation of the species had therefore to be based mainly on the structure of the genitalia.

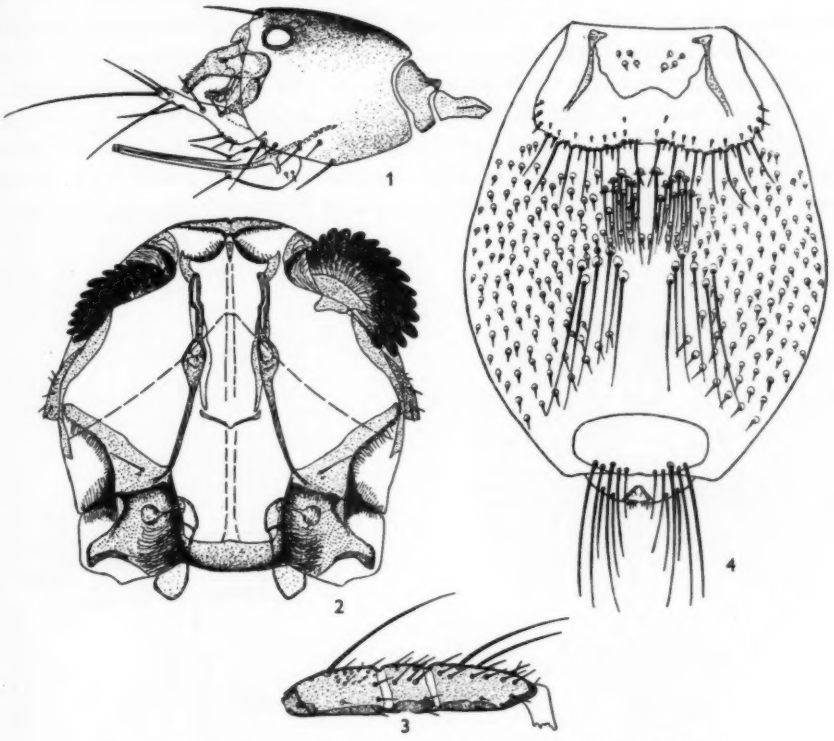
DIAGNOSIS. *Head* laterally compressed, pigmented dorsally, with elliptical, single eyes. Palps with a thick base and a thinner distal part, with a long terminal seta and a few short setae on the thin part and on the ventral surface of the basal thick part. Labella of the labium much longer than the theka, in some species twice as long. *Thorax* roughly pentagonal, narrow anteriorly between the ctenidia. Thoracic ctenidia consisting of thick, curved, blunt spines with secondary striations. Mesonotal space narrow anteriorly, widening posteriorly. Lateral plates of the notopleural sutures present only in the posterior third. Only one large and one minute notopleural seta. Mesopleural sutures broad, directed obliquely forward. Oblique sutures (between meso- and metasternum) well marked. Hairs in front of them much smaller than behind them. Setae at the posterior ventral margin short. Tibiae long, parallel sided, cylindrical, only slightly flattened, with two rings of weaker chitin in the middle and short setae at the proximal margin of the rings. A group of small spines on the dorsal surface of the fore tibia near the base and on the hind tibia near the apical end. *Abdomen*. Tergites 1 and 2 fused. Abdominal ctenidium consisting of thick blunt spines with secondary striations. Post-spiracular sclerite short with a single seta near sternite 1 + 2. Segmentation of the abdomen of the female much reduced, segments 3 to 5 fused into a membrane. Segmentation of the abdomen of the male normal. *Genitalia*. Ventral genital plate of the female divided into halves which bear spines. Dorsal plate vaulted, rounded anteriorly. An anal sclerite of characteristic shape near the genital plates. Aedeagus tubular, with a large endophallus and connected with the phallobase by a connecting membrane which bears numerous teeth ventrally. Sternite 9 (basal arc) narrow, triangular with long anterior process. Claspers straight, thin, covered with spines and hairs. The spines are peglike in some species. A detailed description of the genitalia has been given elsewhere (Theodor, 1952).

1. *Eucampsipoda hyrtlii* Kolenati, 1856 (*Die Parasiten der Chiropteren*, Brünn.)

Length 2·5–3 mm.

Head (Fig. 1). Moderately compressed laterally. Dorsal surface darkly pigmented. The eyes are large, unpigmented, elliptical, situated close to the anterior

margin of the head which bears usually 2, but sometimes 3 or 4 setae between the eyes. Four to 5 moderately long hairs at the posterior part of the ventral margin of the head. Genae bare. Palps thick at the base and thin distally, bearing a long seta at the tip, 4 to 5 hairs on the thin part and a few hairs at the base on the ventral surface. Labella of the labium nearly twice as long as the theka, which has 3 short spines at each side near the posterior edge.

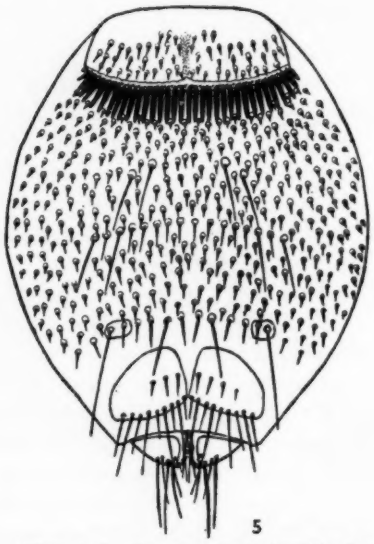


Figs. 1–4. *Eucampsipoda hyrtlii*. 1, head, profile, 2, thorax, dorsal, 3, fortibia, 4, female, abdomen, dorsal.

Thorax (Fig. 2). Roughly pentagonal, anterior edge between the ctenidia less than half the width of the thorax. Median sternal suture narrow, well marked, except for a short indistinct part in the middle. Oblique sutures well marked, slightly curved, joining the median suture at a right angle. The hairs in front of them much smaller than those behind them, except for a row of longer hairs along the base of the ctenidia. These have 16 to 18 large, blunt teeth with secondary striations. The notopleural sutures lie close together anteriorly and diverge posteriorly. Anterior spiracles elliptical, situated at the anterior third of the thorax. Mesopleural sutures well marked, broad and tapering laterally, originating at the posterior third of the notopleural sutures. Lateral plates of the notopleural sutures absent in front of the mesopleural suture. Only one large and one minute noto-

pleural seta present. Tibiae cylindrical, slightly flattened, as long as the femur in the foreleg, slightly shorter than the femur in middle and hindleg. Setae on the tibiae short, except for 3 long ones on the dorsal surface, one near the base and two near the apex (Fig. 3).

Abdomen female (Figs. 4, 5). First tergal plate (which consists of the fused first two tergites) trapezoid, with slightly concave posterior margin and with rounded corners. A row of moderately long and short setae at the posterior margin. One or two rows of short hairs close to the marginal row and at the sides. Two groups of 3 to 5 minute hairs near the anterior border, on tergite 1. Tergites 3 to 5 fused into a membrane, covered with small spines. In the middle of the dorsum two groups of 3 to 6 long setae and in front of them, behind the middle of tergite 1 + 2, two other groups, each of about 10 shorter and thinner setae. The space between and behind the long setae is bare. Tergite 6 broadly elliptical, bare, with a row of 8 to 10 long setae at the hindmargin with sometimes a gap in the middle. In some specimens



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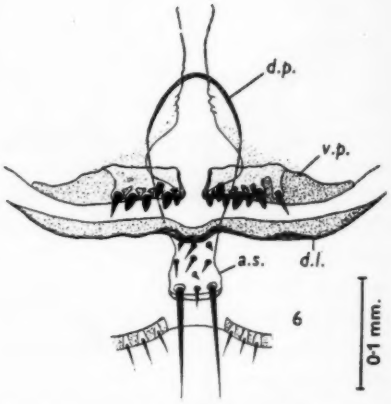
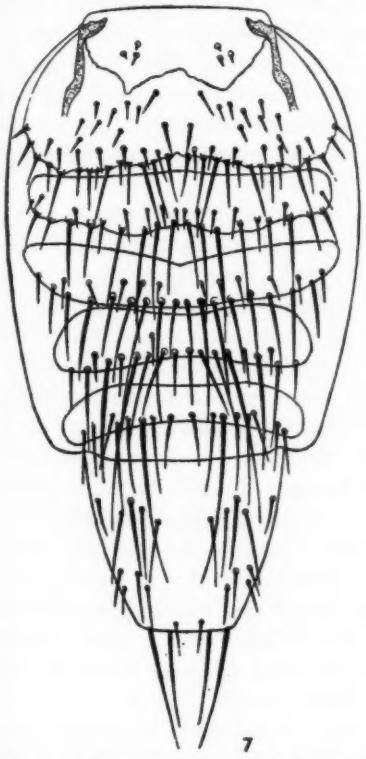


Figs. 5, 6. *Eucampsipoda hyrtlii*. 5, female, abdomen, ventral, 6, female, genital plates. *a.s.*, anal sclerite; *d.l.*, dorsal lip; *d.p.*, dorsal plate; *v.p.*, ventral plate.

1–2 setae on the surface near the marginal row. The median setae are shorter than the lateral ones. Anal segment very short, with a few long setae at the sides of the anus. Sternite 1 + 2 rectangular, short, with a ctenidium of 32 to 36 thick spines and 3 to 4 rows of short setae in its posterior half. Post-spiracular sclerite with a single, moderately long seta near sternite 1 + 2. Abdomen behind the basal sclerite covered with short spines. Four long setae in the middle of sternite 3, two anteriorly and two further posteriorly and farther apart. Sternite 3 is covered with about 7 rows of short spines. The hindmargin of the sternite is indicated by 2 long setae at each side and the other setae of the row are slightly longer than the short spines of the surface. There is a narrow bare space between sternites 3 and 4

in extended specimens. The posterior row of sternite 4 consists of short spines, only two of which in the middle are slightly longer than the others. Two to 3 rows of short spines on the surface. The posterior row of sternite 5 consists of spines slightly longer than the spines of the surface and of 4 long setae, of which 2 at the sides stand with a short seta on a small sclerite. Two to 3 rows of short spines on the surface. Sternite 6 divided into two roughly triangular plates which bear a row of 4 long setae alternating with 4 to 5 short setae at the hindmargin and a transverse row of 3 or 4 short setae in the middle of the surface.



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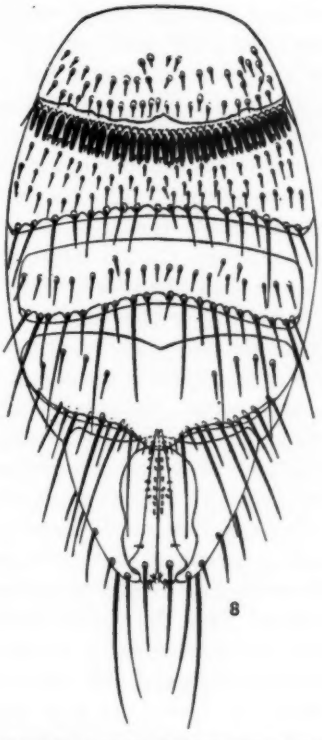

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Figs. 7, 8. *Eucampsipo hyrtlii*. 7, male, abdomen, dorsal, 8, male, abdomen, ventral.

Genital plates (Fig. 6). Ventral plate divided into two wing-shaped halves, each of which bears about 6 short, thick, peglike spines at the inner dorsal corner. Dorsal plate large, rounded anteriorly, longer than broad, vaulted, fused at its posterior edge with the anal sclerite, forming a narrow lip, which covers the genital opening from above. This anal sclerite has a small, more strongly chitinized plate in the middle, which bears 2 longer setae at the end and a few small spines on the surface. This small plate will be referred to as the anal sclerite in short.

Abdomen male (Figs. 7, 8). Tergite 1 + 2 broader than in the female and with 2 to 3 rows of short setae in front of the marginal row. Tergites 3 to 6 narrow with a row of moderately long setae at the posterior margin and a row of short hairs in

the middle of the surface which may be double on tergite 3 and a single row on tergite 4. Tergite 5 with only a few hairs in the middle or bare and tergite 6 bare on the surface. Two to 4 longer setae in the middle of the hindmargin of tergites 4 to 6. Anal segment conical, with some moderately long setae in the posterior half of the dorsal surface and some long setae at the tip. Sternite 1 + 2 as in the female. Sternite 3 narrow with rather short setae posteriorly and 2 to 3 rows of short setae on the surface. Sternite 4 similar, but some of the posterior setae longer and only one row of short setae on the surface which may have a gap in the middle in some specimens. The anterior margin of sternite 4 is straight or slightly convex. Sternite 5 is longer and triangularly produced in the middle of the hindmargin. A row of long and short setae at the sides of the hindmargin. There are a few short thin hairs at the process in the middle and in most specimens 2 short, thick pegs and 2 much smaller ones. In some specimens these pegs are absent. Surface of the sternite bare except for a transverse row of short setae which is broadly interrupted in the middle. Anterior margin indented in the middle.

Genitalia (Figs. 9, 10). Claspers long (0·48 mm.), straight and pointed in dorsal view. In profile, the tip of the clasper is widened and has a short tooth ventrally close to the tip. On the dorsal surface 2 rows of 6 to 8 short, thick pegs from near the tip to about the middle of the clasper. The lateral row begins nearer the tip and the dorsal row reaches further basally. There is sometimes a short additional row between the two others. A few minute spines anteriorly to the dorsal row of pegs. A row of thin hairs at the lateral border of the ventral surface from near the tip to the basal quarter, the hairs being longest near the tip and shorter towards the base of the clasper. A long seta on the dorsal surface near the base. Basal arc (sternite 9) triangular with a long anterior process and a concave posterior margin. Aedeagus cylindrical with oblique anterior opening, 0·43 mm. long, 8 times as long as broad at the base. Parameres small, triangular with 2 small hairs near the anterior margin. Apodeme slightly longer than the aedeagus, thin and surrounded by a sleeve which is as wide as the aedeagus near its junction with it. The aedeagus is connected with the conical phallobase by a membrane which bears ventrally numerous teeth pointing backwards in the extended position. There is a wide endophallus covered with small backwards pointing teeth.

HOST. *E. hyrtlii* has been found so far only on *Rousettus aegyptiacus* and is apparently the only Nycteribiid parasitizing this bat normally. Only a single specimen of *N. pedicularia* and a few specimens of *N. schmidlii* were found among several hundred *Eucampsipoda* examined. A single specimen of *E. hyrtlii* was found in Egypt on *Rhinopoma hardwickei cystops*.

DISTRIBUTION. *E. hyrtlii* has been identified with certainty only from the Middle East (Arabia, Egypt, Palestine and Syria). Its most northern point of distribution is Antioch in southern Turkey which geographically belongs to northern Syria. It is to be expected in Cyprus where *R. aegyptiacus* occurs. It probably occurs in East Africa wherever *R. aegyptiacus* occurs, but no specimens from this area have been available for examination. All specimens of *Eucampsipoda* from tropical Africa examined proved to be *E. africanum* n.sp. Records of *E. hyrtlii* from the Oriental region refer either to *E. latisternum* or to several other new species described below.

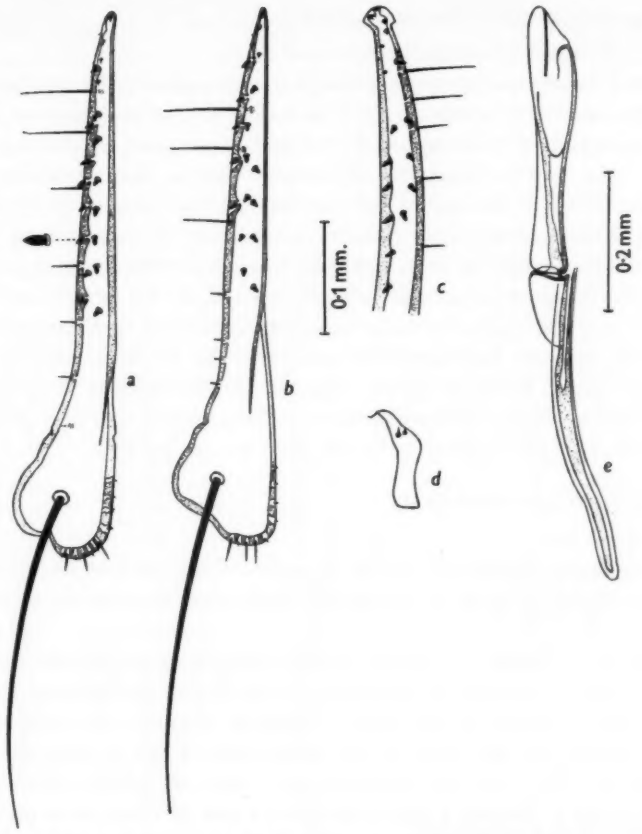


Fig. 9. *Eucampsipoda hyrtlii*, male genitalia: (*a*) clasper, dorsal; (*b*) clasper, dorsal with additional row of pegs; (*c*) clasper, profile; (*d*) paramere; (*e*) aedeagus.

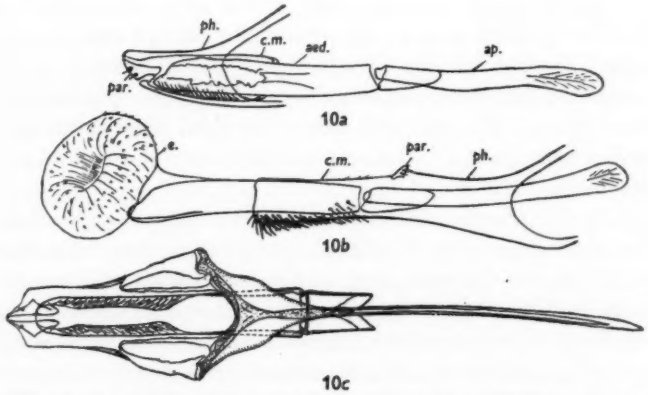


Fig. 10. *Eucampsipoda hyrtlii*, male genitalia: (*a*) resting position, profile; (*b*) extended position (*aed.*, aedeagus; *ap.*, apodeme; *c.m.*, connecting membrane; *e.*, endophallus; *ph.*, phallobase; *pa.*, paramere); (*c*) resting position, dorsal.

2. *Eucampsipoda aegyptia* Macquart, 1851.

Syn.? *N. fitzingeri* Kolenati, 1856.

Macquart's description gives no characters which permit the recognition of the species. Speiser (1901) is already in doubt whether the inclusion of *N. aegyptia* Macq. in the genus *Eucampsipoda* is justified. Macquart neither mentions nor figures the rings on the tibiae. On the other hand, he draws two long processes with setae at the tip at the end of the abdomen of his specimen (a female), which suggests that his specimen was possibly a female of *N. biarticulata*. Kolenati's description of *N. fitzingeri* in 1856 and of *E. aegyptia* in 1863 does not give any good characters for the separation of *E. aegyptia* from *E. hyrtlii*, and it is possible that both species are identical. The only specimens identified as *E. aegyptia* found in any collection were two males in the Museum in Berlin which proved to be *E. hyrtlii*. The host given by Kolenati for *E. aegyptia* (*Asellia tridens*) is unusual for an *Eucampsipoda* which as a rule are parasites of Megachiroptera. The question of the identity of *E. aegyptia* must thus be left open for the present.

3. *Eucampsipoda africanum* n.sp.

Length 2·5–3 mm.

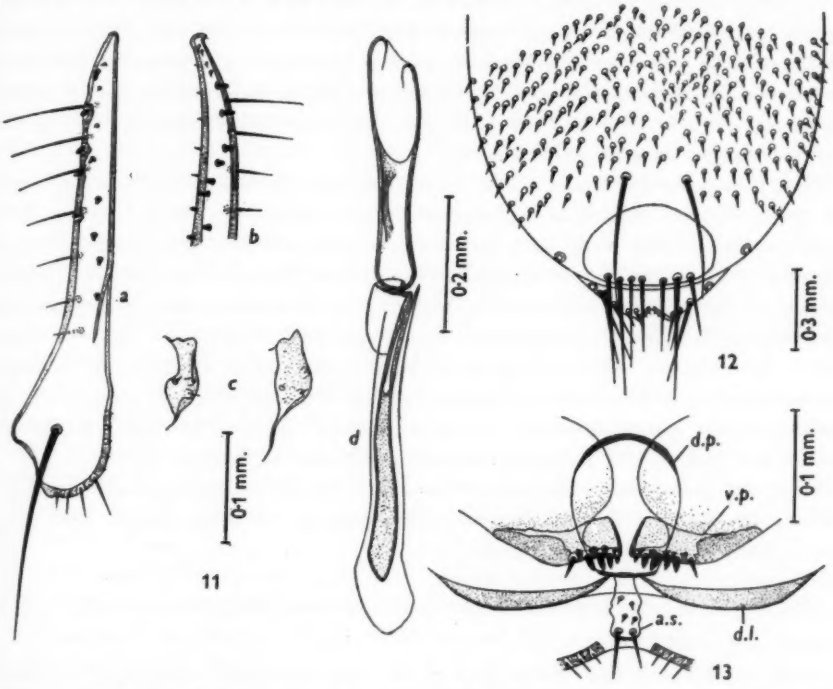
Head and thorax similar to that of *E. hyrtlii*. Four setae at the anterior dorsal margin of the head. Labella of the labium slightly less than twice the length of the theka.

Abdomen male. Tergite 1 + 2 broad with an indentation in the middle of the hindmargin. A row of moderately long setae along it and an irregular row of short setae close to it, mainly at the sides. Abdomen dorsally otherwise much as in *E. hyrtlii*, except that the setae at the hindmargin of the tergites are longer and more numerous. There are two very long setae near the middle of the hindmargin of tergites 5 and 6. Tergite 5 has a continuous row of short setae on the surface and tergite 6 is bare. Sternite 1 + 2 rather short, rectangular, with a ctenidium of 34 to 36 thick, blunt spines and two rows of short setae in the posterior part of the surface. Post-spiracular sclerite short, rectangular with a very short spine near sternite 1 + 2 (rarely double or even triple at one side). Sternites 3 and 4 as in *E. hyrtlii*. The row of short setae on the surface of sternite 4 sometimes interrupted in the middle. Anterior margin of sternites 4 and 5 indented in the middle. Sternite 5 rather short, with a median rounded process posteriorly, a row of long and short setae at the sides and only a few short hairs at the process in the middle. Surface bare, except for one or two short setae at the sides of the surface in some specimens.

Genitalia (Fig. 11). Claspers thin, straight, pointed in dorsal view, 0·43 mm. long. In profile the tip is widened less than in *E. hyrtlii* and the ventral tooth is rounded. A row of 5 to 7 pegs at the outer edge on the dorsal surface from near the tip to about the middle of the clasper and a row of much smaller spines in the middle of the dorsal surface, which are ordinary spines anteriorly and pegs posteriorly. This row reaches further posteriorly than the lateral row. A row of hairs at the outer side on the ventral surface which begins near the 2nd lateral peg. The hairs are longest near the tip of the clasper. A long seta on the dorsal surface near the base.

Basal arc triangular with concave posterior margin. Phallobase broader than in *E. hyrtlii*, aedeagus more strongly chitinized, shorter and broader than in *E. hyrtlii*, 0·38 mm. long, 7 times as long as broad at the base. Sleeve around the base of the apodeme short and wide. Parameres broad, with rounded ventral edge and 3 to 4 small hairs. Spines of connecting membrane much longer than in *E. hyrtlii*, anteriorly nearly as long as the width of the aedeagus.

Abdomen female (Fig. 12). Tergite 1 + 2 similar to that of *E. hyrtlii*. Median setae of hindmargin as long as the others. Dorsum of abdomen covered with short spines which are longer laterally and posteriorly. Only 2 long setae (rarely 3 or 4)



Figs. 11–13. *Eucampsipoda africanum* n.sp. 11, male genitalia: (*a*) clasper, dorsal; (*b*) clasper, profile; (*c*) paramere; (*d*) aedeagus. 12, female, posterior part of abdomen, dorsal. 13, female, genital plates. Letters as in Fig. 6.

near the posterior margin of the area of short spines. The anterior group of long setae is absent. Tergite 6 large, elliptical, bare with a row of 5 to 8 long setae at the hindmargin, usually with a gap in the middle. The median setae are often shorter than the lateral ones. Anal segment very short, with several long setae at the sides of the anus. Sternite 1 + 2 as in the male, but with 3 rows of short setae at the sides of the surface. Ventral surface of the abdomen covered with short spines. The two posterior setae of the four in the middle of sternite 3 only slightly posterior to the anterior setae. The spines of the posterior row of sternite 3 are longer than the spines of the surface, and there are two long setae at each side of the row. Four

setae near the middle of the posterior row of sternite 4 longer than the others, which are also longer than the short spines of the surface, so that the row stands out clearly. The marginal row of sternite 5 consists of moderately long and 4 long setae of which two at the sides stand with a shorter seta on a small round sclerite.

Genital plates (Fig. 13). Ventral plates rather short and broad, with 5 to 6 short thick pegs at the inner dorsal corner. Sometimes an ordinary spine instead of a lateral peg. Dorsal plate broad, rounded, anal sclerite narrow, slightly wider posteriorly, with two longer setae at the end and several short spines on the surface. Dorsal lip broad, produced in the middle.

E. africanum differs from *E. hyrtlii* in the male in minor details of chaetotaxy and in the structure of the genitalia, in the female in the absence of the anterior group of long setae on the dorsum of the abdomen, the position and small number of the setae of the posterior group and in the shape of the genital plates. Some records of *E. hyrtlii* from Ethiopian Africa probably refer to *E. africanum* which seems to be widely distributed.

HOSTS AND DISTRIBUTION. *East Africa.* Kenya, Bahati cave, *Rousettus leachi*, 16 spec. Types. (Garnham) Nakuru, 14 spec. (Zumpt). A. E. Sudan, Jebel Goumia, Acholi Mts. near Torit, *R. leachi*, 16 spec. (Owen). Tanganyika, Pemba Island near Zanzibar, *R. leachi*, 6 spec. (Bequaert). Makoa, Kilimanjaro, *R. leachi*, 4 spec. (Ergebnisse der Deutschen Zoologischen Ost Africa Expedition, Gruppe Lindner, 1951–2, No. 6). Nyasaland, Karonga, 3 spec. (Davey). *Belgian Congo.* Banza Nfinda cave, Tamba Tamba cave, *R. aegyptiacus*. Kakontwé, Katanga, Atshokari cave, Yolohafiri cave, Irumu. Nyanza. Pteropidae, *c.* 100 spec. (Leleup). *South Africa.* Cape province, Knysna, *Eidilon helvum*, 14 spec. Rothschild collection. Salem near Grahamstown, *Rousettus leachi*, 3 spec.

Type and paratypes in the British Museum (Nat. Hist.), paratypes in the South African Institute of Medical Research, the Museum du Congo Belge, Tervueren, the Hebrew University, Jerusalem.

4. *Eucampsipoda latisternum* Schuurmans Stekhoven, 1939 (*Capita Zoologica*, **8**, 1.)

Length 2·5–2·75 mm.

Head as in *E. hyrtlii*. Four setae at the anterior dorsal margin of the head. Labella of labium nearly twice as long as the theka. Thorax as in *E. hyrtlii*. Posterior part of sternum only slightly wider than in *E. hyrtlii*.

Abdomen male. Dorsal surface similar as in *E. hyrtlii*. One or two rows of short setae on the surface of tergites 3 to 5. Only one row on the surface of tergite 6 which is broadly interrupted in the middle. Sternite 1 + 2 with a ctenidium of 32 to 35 spines. Post-spiracular sclerite broad with a very short seta. Sternite 4 with a broadly interrupted row of short setae on the surface. Sternite 5 bare or with 1 to 3 short setae at the sides of the surface. Hindmargin triangularly produced with long setae at the sides and short hairs at the process in the middle.

Genitalia (Fig. 14). Claspers straight, rather broad and short (0·3 mm.) with a short blunt tip. On the outer edge, near the tip, a row of short, strong spines (not pegs) which are shorter and thicker near the tip and become gradually longer and thinner basally. This row is sometimes double basally and some of the spines

stand on the dorsal surface. Also on the dorsal surface, near the tip, a row of several very small spines. A row of setae at the lateral edge of the clasper from near the tip to the basal third. These setae are short near the tip and longest in the middle. A large seta dorsally near the base. Basal arc triangular. Aedeagus 0·35 mm. long, similar to that of *E. hyrtlii*, but broader, 6 to 7 times as long as wide at the base. Parameres long and narrow, triangular, with a single small hair at the ventral edge. Teeth of the connecting membrane rather short.

Abdomen female (Figs. 15, 16). Tergite 1 + 2 as in *E. hyrtlii*, but only with a single row of short setae close to the marginal row. The anterior group of long setae is represented by 8 to 10 fine hairs behind the middle of tergite 1 + 2 arranged in two transverse rows. Two groups of 3 to 5 long setae in the middle of the dorsum which sometimes form diagonal rows. Short spines between and behind these setae. Tergite 6 very narrow, bare, with a row of 10 to 12 long setae at the hindmargin, sometimes with a small gap in the middle. Anal segment short, with 3 to 6 setae at the sides of the anus. Sternite 1 + 2 with 3 rows of short setae on the surface, of which those of the anterior row are longest, and a ctenidium of 36 teeth. The short spines of the ventral side of the abdomen rather strong. The marginal rows of sternites 3 to 5 consist of longer setae than the short spines of the surface. The arrangement of longer setae similar to that of *E. africanum*. Sternite 6 divided into two triangular plates, each of which bears 4 to 5 long setae alternating with 4 to 5 short setae at the hindmargin and a row of 4 to 5 rather strong, moderately long setae in a transverse row in the middle of the surface.

Genital plates (Fig. 17). Ventral plates broad, rectangular or broadly triangular, with a row of 5 to 7 pegs at the inner dorsal corner. Sometimes an ordinary spine instead of a lateral peg. The two rows of pegs form an obtuse angle and are visible externally, as the lip which covers the genital opening is reduced to two lateral flaps which do not cover the ventral plates. Dorsal plate broad, nearly square. Anal sclerite absent.

E. latisternum differs in the male from *E. hyrtlii* by the form of the claspers. In the female by the arrangement of the long setae on the dorsum of the abdomen, the form of tergite 6, the form of the genital plates and the absence of the anal sclerite. The specimens from Ceylon described and figured by Scott (1914) as *E. hyrtlii* are *E. latisternum*. This appears also from the description of the claspers of the male which, as Scott states, have 'short thorn bristles along the outer margin towards the apex'. The specimens from Ceylon which were used by Ferris for comparison with *E. philippinensis* and considered by him as *E. hyrtlii* were also *E. latisternum*, which is the only species known with long spines and hairs on the claspers.

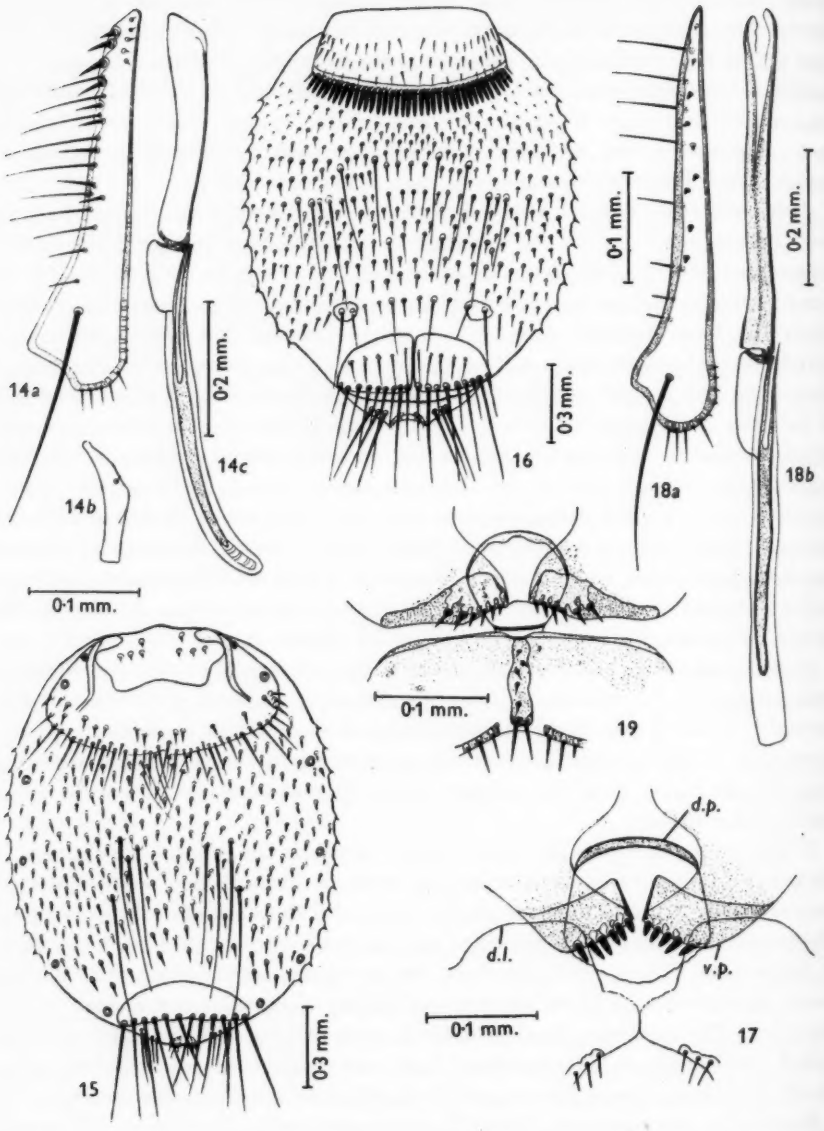
Hosts and distribution. Java (*R. amplexicaudatus*.). Farm caves, Tenasserim, Burma (*Pteropus* sp.). Ceylon (*R. seminudus*).

5. *Eucampsipoda madagascarensis* n.sp.

Length 2·5–3 mm.

Head and thorax similar to *E. hyrtlii*.

Abdomen of the male as in *E. hyrtlii*, except that the short setae of the surface of the tergites are more numerous and tend to form a double row in the middle.



Figs. 14–17. *Eucampsipoda latisternum*. 14, male, genitalia: (*a*) clasper; (*b*) paramere; (*c*) aedeagus. 15, female, abdomen, dorsal. 16, female, abdomen, ventral. 17, female, genital plates. Letters as in Fig. 6.

Figs. 18, 19. *Eucampsipoda madagascarensis* n.sp. 18, male, genitalia: (*a*) clasper; (*b*) aedeagus. 19, female, genital plates.

The row of short setae on sternite 4 is continuous and double at the sides in some specimens. The anterior margin of sternite 4 is straight.

Genitalia (Fig. 18). Claspers slightly shorter than in *E. hyrtlii* (0·38 mm.) and tapering more uniformly. An irregular row of 7 to 9 very small spines and pegs from the tip to the basal third of the clasper on the dorsal side. The first 2 or 3 near the tip are ordinary spines and the others are pegs which become larger towards the base. The pegs are about half as large as those of *E. hyrtlii*. A row of hairs laterally on the ventral surface which are longer near the tip and shorter basally. A long seta dorsally near the base. Phallobase narrow. Aedeagus very long and narrow, 0·52 mm. long, 12–14 times as long as wide at the base, straight or slightly curved and with a rounded tip. Sleeve around the base of the apodeme long and narrow, not much wider than the apodeme, which is only slightly longer than the aedeagus. Endophallus with only a few narrow teeth. Teeth of connecting membrane rather short. Parameres small, pointed, narrowly triangular.

Abdomen female. Tergite 1 + 2 with a row of long setae posteriorly and a single irregular row of short setae, which is interrupted in the middle, close to the marginal row. There are two groups of long setae on the dorsum of the abdomen. Both these groups are not divided into lateral parts as in *E. hyrtlii*. The anterior group consists of about 22 long thin hairs arranged in 2 or 3 transverse rows. The posterior group consists of 7 to 10 long setae. There are 2 long setae between the anterior group of hairs and the posterior group of setae. The spines of the dorsum are markedly longer posteriorly and the posterior row consists of spines longer than the others. Tergite 6 small, elliptical with 6 to 8 long setae at the hindmargin of which those in the middle are shorter than the others. The arrangement of short spines and long setae on the ventral surface similar to that of *E. africanum*, i.e. the 4 setae on sternite 3 stand close together in the longitudinal direction and the setae of the marginal row of sternite 4 are longer than the short spines of the surface. Anal segment rounded, longer and more projecting than in *E. hyrtlii* with a few long setae at the sides of the anus. Sternite 6 consisting of two rather small triangular or rounded plates with 4 long setae alternating with 4 shorter setae a short distance before the hindmargin and a transverse row of 2 to 3 short setae in the middle of the surface.

Genital plates (Fig. 19). Ventral genital plates triangular with a row of about 6 short spines (not pegs) at the dorsal margin. Dorsal plate broadly rounded, as broad as long. Anal sclerite long, narrow, parallel sided with two longer setae at the end and several short spines on the surface. Dorsal lip narrow, smoothly rounded.

E. madagascarensis is closely related to *E. hyrtlii* from which it differs mainly in the structure of the genitalia and the smaller number of setae on tergite 6 of the female. Speiser (1908) recorded *E. hyrtlii* from the Comoro Islands. In view of the occurrence of the above new species in Madagascar, it is likely that his specimens also belong to this species and his material should be re-examined.

Host and distribution. Fort Dauphin, Mananteira, Madagascar, 28 November 1948. 4 ♂♂, 4 ♀♀ on *Rousettus madagascarensis*. (coll. H. Hoogstraal). Type and paratypes in the U.S. National Museum. Paratypes in the Chicago Natural History Museum and the Hebrew University, Jerusalem.

6. *Eucampsipoda penthetoris* n.sp.

Length 2·25–2·5 mm.

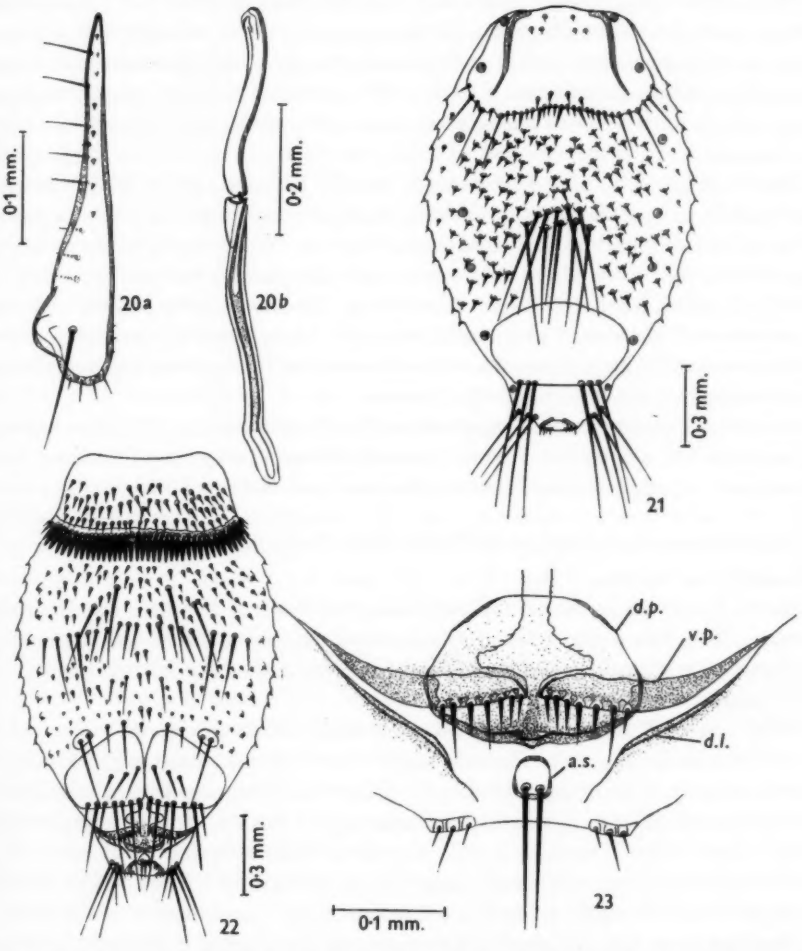
Head with only 2 setae at the anterior dorsal margin. Eyes smaller than in *E. hyrtlii*. Labella of labium 1·5 times as long as theka.

Thorax. The short spines in front of the oblique sutures of the sternal plate much smaller than the spines behind them, except the row along the anterior border of the oblique sutures, which, however, are not as large as the spines behind the sutures. The minute spines in front of the sutures do not cover the whole plate, but leave an area in the middle bare. Notopleural sutures close together anteriorly, bending sharply outward posteriorly. Large notopleural seta strong and relatively short. Mesopleural sutures narrow and curved. All setae on the legs rather short. The long setae on the dorsal surface of the tibiae less than twice the width of the tibia. The rings on the tibiae closer to the base than in *E. hyrtlii*, the apical ring at about the middle of the tibia.

Abdomen male. Tergite 1 + 2 slightly concave posteriorly and a row of long and short setae at the sides and only short setae in the middle of the hindmargin. There is an irregular row of short setae close to the marginal row. Tergite 3 with a row of moderately long and short setae posteriorly and a double row of short setae on the surface. Tergite 4 similar, but the marginal row does not reach the sides and there is only a single row of 6 to 8 short setae on the surface. Tergite 5 and 6 bare, with longer setae at the hindmargin. Two to 4 setae near the middle longer than the others, but shorter than in the other species. Anal segment narrow, shaped like a truncated cone, with a few moderately long setae at the sides of the posterior half and at the tip. Sternite 1 + 2 with a ctenidium of 36 to 38 teeth and 3 rows of short spines in the posterior half of the surface. Post-spiracular sclerite rather long and narrow, with a short spine near sternite 1 + 2 and pointed at the free end. Sternite 3 with moderately long and short setae at the hindmargin and 2 to 3 rows of short setae on the surface. Sternite 4 with widely spaced, alternating moderately long and short setae at the hindmargin and a broadly interrupted row of short setae on the surface. In some specimens only 1 to 2 such setae at each side and the marginal row with a gap in the middle. Anterior margin of the sternite slightly indented. Sternite 5 with the anterior margin broadly indented in the middle. Hindmargin only slightly convex, without the median process present in most other species and with alternating long and short setae at the sides and only short fine hairs in the middle. Surface bare except for 2 to 4 short setae in a transverse row at the sides.

Genitalia (Fig. 20). Claspers 0·35 mm. long, wide in the basal half, tapering towards the middle, much narrower, nearly parallel sided, in the apical half, which tapers to a long point. A row of fine hairs laterally on the ventral side from the tip to the basal third. A strong, moderately long seta dorsally near the base. A row of minute spines from the tip to about half the length of the clasper. Basal arc narrow, triangular. Aedeagus short (0·3 mm.), very narrow, slightly curved, 9 to 10 times as long as wide at the base, slightly tapering to a rounded tip. Endophallus small, without teeth. Parameres small, triangular with a minute hair. Teeth of the connecting membrane fine and short.

Abdomen female (Figs. 21, 22). Long, elliptical in gravid females, not rounded or truncated as in *E. hyrtlii*. Tergite 1 + 2 as in male. Dorsum of abdomen covered with small spines which are longer posteriorly. In the middle of the dorsum a group of 10 to 14 long setae arranged in 2 to 3 transverse rows. Behind this row



Figs. 20–23. *Eucampsipoda penthetoris* n.sp. 20, male genitalia: (*a*) clasper; (*b*) aedeagus. 21, female, abdomen, dorsal. 22, female, abdomen, ventral. 23, female, genital plates. Letters as in Fig. 6.

the abdomen is bare. The anterior group of long setae is absent. Tergite 6 broadly elliptical or rectangular, bare on the surface, with 2 groups of 3 long setae at the sides of the hindmargin, leaving a broad gap in the middle. Spiracles of segment 6 outside the tergite, but close to its sides. Anal segment longer than in *E. hyrtlii*, shaped like a broad, truncated cone or rounded with some long setae at the sides of the anus. Sternite 1 + 2 as in the male with a ctenidium of 42 to 44 teeth and 3 to

4 rows of short setae on the surface. Sternite 3 with 6 to 8 rows of short spines and 4 long setae in the middle. A row of moderately long and short setae at the hindmargin. Two setae at each side longer than the others. Sternite 4 and 5 with 2 rows of spines on the surface, which are much shorter and more widely spaced than on sternite 3. Two longer setae in the middle of the marginal row and 2 shorter setae farther laterally. Four long setae in the marginal row of sternite 5 of which two near the sides are longest and stand with a short one on an elliptical plate, which is larger than in the other species. Sternite 6 divided into two large, roughly triangular plates which have a row of 3 long setae alternating with about 4 short setae at the hindmargin and a transverse row of 2 to 3 short setae in the middle of the surface.

Genital plates (Fig. 23). Dorsal plate broadly rounded, much wider than long. Ventral plates long and narrow, curved, triangular with a row of 6 to 8 rather long setae at the inner part of the dorsal edge, which are shorter towards the middle and longer laterally. These plates are visible externally as they are not covered by the dorsal lip, which is reduced to two lateral flaps. The anal sclerite is short and broad, does not reach the dorsal plate and bears only 2 long setae at the end.

E. penthetoris clearly differs from the other species in chaetotactic characters and the structure of the genitalia in both sexes.

HOST AND DISTRIBUTION. Singapore, on *Penthethor lucasi*. 2 ♂♂. Type and paratype: British Museum (Nat. Hist.). Sarawak, Borneo. 14 spec. on *Penthethor lucasi*. Paratypes. Chicago Natural History Museum and Hebrew University.

7. *Eucampsipoda philippinensis* Ferris, 1924 (*Philipp. J. Sci.* **24**, 73).

Length 2–2·75 mm.

Head. Eyes very small, about half the size of those of *E. hyrtlii* and most other species. Only two setae at the anterior dorsal margin of the head. Terminal seta of the palps not much longer than the palp itself. Labella of labium twice as long as the theka.

Thorax as in *E. hyrtlii*. Mesonotal space rather wide anteriorly.

Abdomen male (Fig. 24). Dorsally similar to *E. hyrtlii*. Anal segment long and narrow, nearly cylindrical. Sternite 1 + 2 short, rectangular with a ctenidium of about 30 teeth and 2 to 3 rows of short setae on the surface. Post-spiracular sclerite with a short spine. Sternite 3 with a uniform row of rather short setae at the hindmargin and 3 rows of short spines on the surface which are longer laterally. Sternite 4 with straight or slightly convex anterior margin and a continuous row of short setae on the surface which is narrowly interrupted in the middle in some specimens. Sternite 5 with straight anterior margin, not very strongly produced posteriorly, with a row of long and shorter setae at the sides and a group of 6 to 8 strong, short spines at the apex. The median spines smaller than the lateral ones. A broadly interrupted transverse row of short setae in the middle of the surface.

Genitalia (Fig. 25). Claspers long (0·42 mm.), straight, narrow, with an obliquely truncated tip. A dorsal row of 8 to 10 pegs from near the tip to the basal third. They are smaller near the tip and become larger basally. There is a second row of pegs at the lateral edge, which stand more closely near the tip and which does not reach as

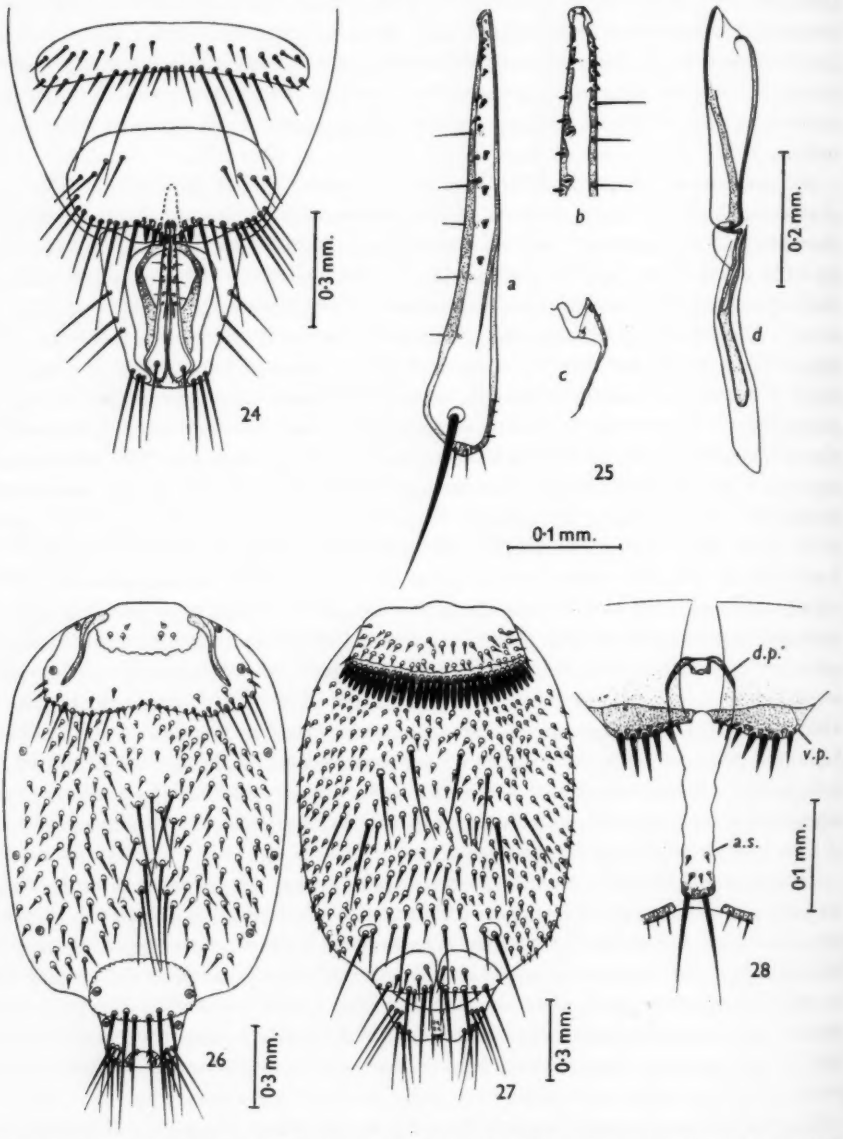
far basally as the dorsal row. This row is inserted on the ventral surface. A row of fine hairs from the apical to the basal third on the ventral surface laterally. A long seta dorsally near the base. Phallobase conical. Aedeagus 0·33 mm. long, 6 times as long as wide at the base, markedly wider apically. Sleeve around the base of the apodeme very short. Apodeme slightly longer than the aedeagus. Spines of the connecting membrane rather short and thick. Parameres with a long ventral posterior process, an anterior ventral pointed and a dorsal rounded process. A deep concavity between the two processes. Two minute spines at the ventral edge and another in the middle of the inner edge. Endophallus large, covered with short teeth.

Abdomen female (Figs. 26, 27). Tergite 1 + 2 with concave hindmargin. The row of setae at the hindmargin consists of 5 to 6 moderately long setae alternating with short setae at the sides and only short setae in the middle half. Surface bare except for a few short setae near the marginal row. Dorsum of the abdomen covered with short spines which become longer posteriorly. A longitudinal group of 10 to 13 long setae in the middle of the dorsum. This group begins at the anterior third of the spinose area and there are 6 to 8 rows of short spines in front of it. It does not reach the posterior margin of the spinose area and the setae stand not farther apart posteriorly than anteriorly. The setae are shorter than in most other species. Tergite 6 elliptical, bare, with 6 to 8 long setae at the hindmargin. The spiracles of segment 6 lie inside the tergite and not, as in the other species, in the connective membrane at the sides of the tergite. Sternite 1 + 2 more rounded anteriorly than in the male with a ctenidium of 28 to 30 spines. The marginal rows of sternites 3 to 5 are well developed. There are 4 long setae in the middle of sternite 3 and two strong setae at each side in the marginal row. The median seta of these two is stronger than the lateral seta. The other setae of this row are longer than the short spines of the surface. The marginal row of sternite 4 has two moderately long and several shorter setae in the middle. Marginal row of sternite 5 with 4 long setae of which those at the sides stand on a small plate together with a short seta. Sternite 6 divided into two triangular plates with 3 long and 3 to 4 short setae at the hindmargin and a transverse row of 2 to 3 short setae in the middle of the surface. Anal segment rather long, with some long setae at the sides of the anus and another row of 3 to 4 setae markedly farther basally.

Genital plates (Fig. 28). Ventral plates broadly triangular, broader laterally than medially, with a row of 6 to 8 spines which are longer than the width of the plate at the sides and shorter in the middle. The ventral surface of the anal segment is hollowed out and there is no dorsal lip. The dorsal plate is small, rounded and has an area of thinner chitin near the anterior rim. It is fused with the long anal sclerite into a spoon-shaped structure. The anal sclerite is very long and narrow, with 2 long setae at the end and 2 to 8 short spines on the surface. The ventral plates and their setae are visible externally as there is no dorsal lip.

E. philippinensis is well characterized by a number of characters. The setae in the middle of the hindmargin of tergite 1 + 2 of the female are short. The spiracles of segment 6 lie inside the tergite. The dorsal lip of the genital opening is absent and the genital plates are different from those of all other species known. In the

male the presence of a group of spines in the middle of the hindmargin of sternite 5 distinguishes it from the other species described here except *E. hyrtlii* and claspers and parameres are also of a characteristic form. The drawing of the female by Ferris permits the recognition of the species with certainty. The male figured by



Figs. 24–28. *Eucampsipoda philippinensis*. 24, male, abdomen, posterior part, ventral. 25, male genitalia: (*a*) clasper, dorsal; (*b*) clasper, profile; (*c*) paramere; (*d*) aedeagus. 26, female, abdomen, dorsal. 27, female, abdomen, ventral. 28, female, genital plates. Letters as in Fig. 6.

Ferris, however, probably belongs to the species described here as *E. sundaicum*, which also occurs in the Philippines.

HOSTS AND DISTRIBUTION. Montalban, Rizal province, Luzon, Philippines, from undetermined bat. (Ferris). Kotabato, Mindanao, Philippines, on *Eonycteris robusta* (30 spec.). On *Hipposideros diadema griseus* (38 spec.) 26–27 December 1946 (coll. Werner). Chicago Natural History Museum.

The occurrence of this species on an insectivorous bat is exceptional for the genus.

8. *Eucampsipoda sundaicum* n.sp.

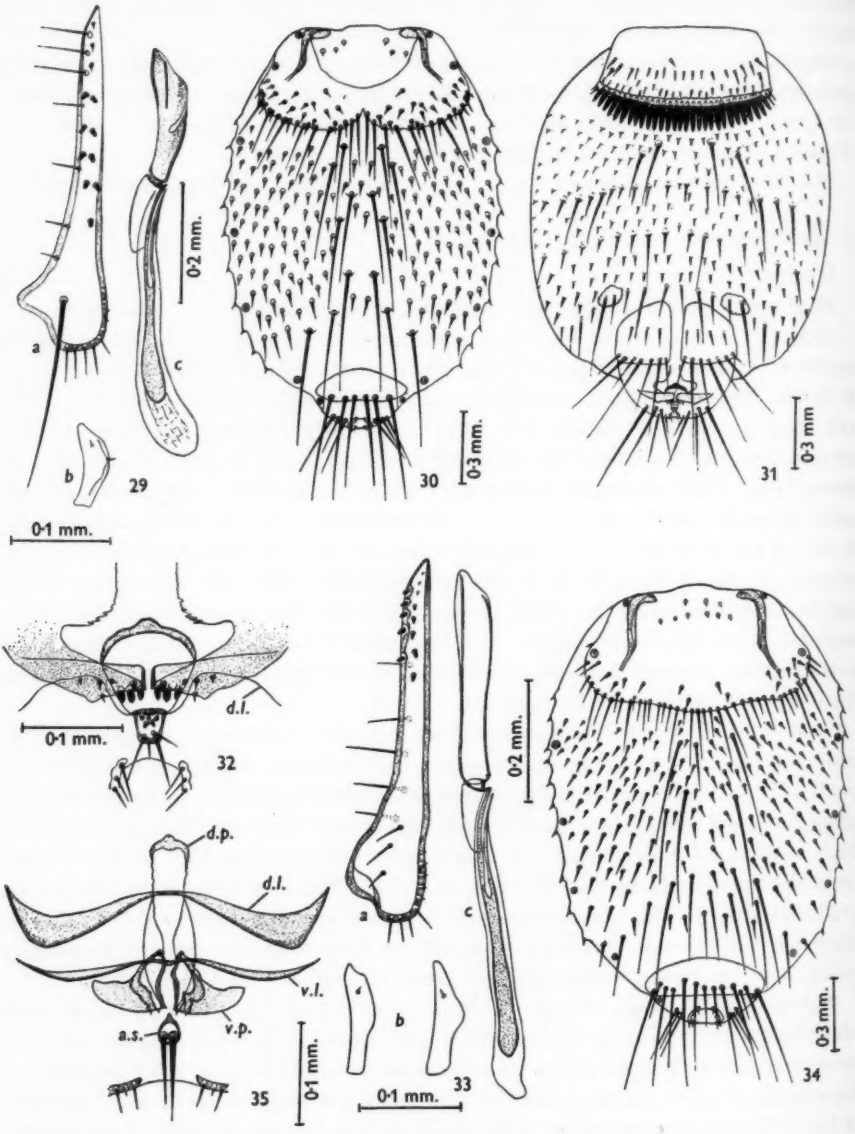
Length 2·5–3 mm.

Head and *thorax* as in *E. hyrtlii*. Labella of labium twice as long as theka.

Abdomen male. Tergite 1 + 2 deeply indented posteriorly, with a dense row of moderately long setae which are longest in the middle. Tergites 3 and 4 as in *E. hyrtlii*. Tergite 5 with a continuous row of moderately long setae on the surface and tergite 6 with a similar row which is broadly interrupted in the middle. Sternite 1 + 2 with a ctenidium of 28 to 30 teeth and with 2 rows of short setae on the surface. Post-spiracular sclerite with a very short spine. Sternite 4 with the anterior margin indented to form a wide obtuse angle or indented in the middle only. A row of longer setae at the sides and of shorter setae in the middle of the hindmargin. A row of short setae on the surface which is broadly interrupted in the middle. Sternite 5 with the anterior margin also indented to form an obtuse angle, moderately produced posteriorly, with long setae at the sides and fine short hairs at the median process. A transverse row of a few short setae laterally on the surface. Anal segment rather broadly rounded.

Genitalia (Fig. 29). Claspers rather short (0·3 mm.), narrow, tapering to a point, with two rows of 5 to 7 thick, large pegs from the apical to the basal third and a few small, pointed spines near the tip. A row of hairs at the lateral edge from the tip to about the basal third. These hairs are inserted on the ventral surface of the clasper. They are longest near the tip. A long seta on the dorsal surface near the base. Basal arc narrow, triangular. Phallobase short, conical. Aedeagus short and wide (0·23 mm. long), 5 times as long as wide at the base and markedly wider distally. Apodeme rather wide and sleeve around its base long and broad. Parameres broad, with rounded ventral edge and blunt tip and with 1–2 short hairs.

Abdomen female (Figs. 30, 31). Tergite 1 + 2 with deeply indented hindmargin which bears a row of long setae of which those in the middle are longest. The long setae alternate with short spines laterally. Two to 3 rows of short setae laterally on the surface, close to the marginal row and only a few setae in the middle. Dorsum of the abdomen covered with short spines which become gradually longer posteriorly, the spines of the last row being more than twice as long as those of the first row. The group of long setae begins immediately behind tergite 1 + 2 and reaches to the posterior margin of the spinose area. The setae are very long, 10 to 17 in number, and form two irregular longitudinal rows. The group as a whole is markedly broader posteriorly where the setae sometimes form transverse rows of four. The abdomen behind the long setae is bare. Tergite 6 narrow, bare, generally with 4 to 6 long setae at the hindmargin, rarely with 3 or 8 setae. Anal segment



Figs. 29–32. *Eucampsipoda sundaicum* n.sp. 29, male genitalia: (*a*) clasper; (*b*) paramere; (*c*) aedeagus. 30, female, abdomen, dorsal. 31, female, abdomen, ventral. 32, female, genital plates. Letters as in Fig. 6.

Figs. 33–35. *Eucampsipoda inermis* n.sp. 33, male genitalia: (*a*) clasper; (*b*) paramere; (*c*) aedeagus. 34, female, abdomen, dorsal. 35, female, genital plates. *v.l.*, ventral lip; other letters as in Fig. 6.

short, with several long and short setae at the sides of the anus. Sternite 1+2 rectangular with a ctenidium of 30 to 33 very thick spines and 2 to 3 rows of short setae on the surface. The marginal rows of sternites 3 to 5 well marked. Four long setae in the middle of sternite 3 and 2 long setae at each side in the marginal row, the other spines of which are longer than the spines of the surface. Marginal row of sternite 4 with 2 long setae near the middle and marginal row of sternite 5 with 4 long setae of which those at the side stand on a small plate together with a short seta. Sternite 6 divided into 2 triangular plates which bear 3 long setae alternating with 4 shorter setae at the hindmargin and a transverse row of 3 to 4 short setae on the surface.

Genital plates (Fig. 32). Ventral plates narrow, triangular, with 3 to 4 short, thick pegs near the inner dorsal corner and a row of much smaller ordinary spines on the surface. Sometimes there is a small peg in this row. Dorsal plate broad and rounded anteriorly, sometimes with a process in the middle. Anal sclerite short and square or tapering slightly posteriorly, with 2 or 3 long setae at the end and 7 to 10 small pegs or spines on the surface. The dorsal lip is divided into two lateral flaps, leaving the ventral plates visible externally.

E. sundaicum resembles *E. philippinensis* in some respects, e.g. the number of long setae on tergite 6 of the female, but differs in the structure of the genitalia in both sexes and in the chaetotaxy of the abdomen in the female. The marginal row of tergite 1+2 consists of long setae and the long setae of the dorsum are arranged differently. The spiracles of segment 6 lie outside the tergite. The male described by Ferris as *E. philippinensis* probably belongs to *E. sundaicum* to judge from the drawing of the claspers and of sternite 5 which does not show the group of spines characteristic for *E. philippinensis*.

Hosts and distribution. Tenasserim, Burma on *Pteropus* sp. Type and paratypes: Zoological Museum, Genoa. Madras, India on *Cynopterus sphinx*. Balighi, Sumatra on *Eonycteris spelaea*. British Museum (Nat. Hist). Nazareto cave near Calapan, Mindanao and Tanabag, Puerto Princesa, Palawan Island, Philippines on *E. spelaea glandifera*. Batu caves, Selangor, Malaya on *E. spelaea*. Chicago Natural History Museum.

9. *Eucampsipoda inermis* n.sp.

Length 2·5–3 mm.

Head and *thorax* as in *E. hyrtlii*. Labella of labium 1·5 times as long as theka.

Abdomen male. Tergite 1+2 with a dense row of moderately long setae at the hindmargin. Tergites 3 to 5 with a double row of short setae on the surface, tergite 6 with a single row. Two long setae in the middle of the marginal row of tergite 3, four such setae on tergites 4 to 6. Sternite 1+2 with a ctenidium of 36 spines. Post-spiracular sclerite with a short spine. The short setae on the surface of sternite 4 are arranged in a row which is sometimes double laterally, single in the middle or there is a broad interruption in the middle. Sternites 4 and 5 are indented in the middle of the anterior margin. Sternite 5 bare, triangularly produced in the middle posteriorly. Only 3 out of 38 specimens in one batch had 1 or 2 short setae laterally on the surface.

Genitalia (Fig. 33). Claspers similar to those of *E. sundaicum*, but longer (0·38 mm). The pegs begin close to the tip and do not reach beyond the middle of the clasper. The lateral row consists of 5 to 6 larger pegs and the pegs of the dorsal row (4 to 6) are small near the tip and larger basally. A row of hairs begins at the 4th or 5th lateral peg from the tip and reaches to the basal third of the clasper, the hairs increasing in length towards the base. (In *E. sundaicum* there are several hairs near the tip, distal to the beginning of the rows of pegs.) There is no long seta on the dorsal surface near the base as in the other species described, but one to 3 short setae. Basal arc narrow, triangular. Phallobase narrow, nearly cylindrical. Aedeagus long and narrow (0·35 mm.), 8 to 9 times as long as wide at the base. Apodeme long and narrow with a narrow sleeve around the base. Parameres small, triangular with a minute hair. Teeth of the connecting membrane rather short and thick.

Abdomen female (Fig. 34). Tergite 1 + 2 with a row of moderately long and short setae posteriorly. The setae near the middle longer than the others. A single or double row of short setae close to the marginal row laterally and only a few such setae in the middle. Arrangement of the long setae on the dorsum similar to that of *E. sundaicum*, but the two longitudinal rows stand farther apart and the setae often form transverse rows of four posteriorly. The area between the anterior long setae is covered with small spines. Tergite 6 narrow, bare, with 12 to 14 long and shorter setae posteriorly. Anal segment very short with several long setae at the sides of the anus. The marginal rows of sternites 3 to 5 are similar to those of *E. sundaicum*, but the 2 setae in the marginal row of sternite 4 are only about twice as long as the others. Sternite 6 consists of two triangular plates with 4 long and 5 short setae at the hindmargin and a transverse row of 3 to 4 short setae on the surface.

Genital plates (Fig. 35). Ventral plates triangular, with only 2 to 3 very small ordinary spines near the inner dorsal corner. Dorsal plate very narrow, twice or 3 times as long as wide. Anal sclerite small, drop shaped, with only two long setae, not reaching the base of the dorsal lip, which is uniformly rounded and very broad. There is also a similar ventral lip. Both lips close above the genital opening.

E. inermis resembles *E. sundaicum* in some respects. It differs, however, in details of chaetotaxy in both sexes, in the chaetotaxy of the claspers, particularly the absence of the long seta on the dorsal surface, the number of the long setae on tergite 6 in the female and in the structure of the genitalia in both sexes.

HOSTS AND DISTRIBUTION. Soribao, Borongan, Samar Island, Philippines 'on dogfaced bat'. Type and paratypes: British Museum (Nat. Hist.). Tjineam, Java. Vienna Museum. Luangbay cave, Davao, Mindanao, on *R. amplexicaudatus* and *Eonycteris spelcea glandifera*. Siaton, Negros Island on *R. amplexicaudatus*. Nazareto cave, Calapan, Mindanao. Tawang cave, S'amal Island, Davao, Philippines. Paratypes. Chicago Natural History Museum and Hebrew University.

Key to the species of Eucampsipoda

Males

The key is based mainly on the structure and the chaetotaxy of the claspers which are visible in alcohol material. The peglike spines on the claspers should be examined under a high magnification and best in balsam preparations. A peg is a barrel-shaped, short spine, generally thicker in the middle than at the base and as a rule not more than 2 or 3 times as long as thick, with a rounded or blunt tip. There are transitions from spines to pegs and there are sometimes pegs in one specimen and spines in another in the same position. Pegs and spines are often seen foreshortened and it is sometimes difficult to distinguish between a small peg and a spine. The aedeagus has to be dissected out after treatment in KOH and mounted in side view. A preparation of the whole phallobase with the aedeagus *in situ* is equally good. Dissection of the genitalia is a delicate operation and not always successful and the present key is therefore not as easily applicable as would be desirable, but no external characters were found which gave an easier or safer means of identification. Some chaetotactic differences are mentioned in the descriptions and some are used in the key, but most of them are so small or variable that they are often only apparent if sufficient material of several species is available for comparison. The length of clasper and aedeagus varies to a certain extent within the species and the figures given are the average of measurements of a number of specimens.

1. A group of short spines at the apex of sternite 5. 2
— Only thin hairs at the apex of sternite 5. 3

2. A row of 6 to 8 strong, short spines at the apex of sternite 5 (Fig. 24). **E. philippinensis** Ferris
— Only 2 larger and one or 2 much smaller such spines at the apex of sternite 5. Middle East (Fig. 8).* **E. hyrtlii**

3. Claspers narrow and tapering to a point. Peglike spines on the dorsal surface 4
— Claspers broad throughout or in basal half. Only ordinary spines on the dorsal surface. 8

4. Pegs very small, arranged in a single irregular dorsal row of 7 to 9 pegs. Aedeagus very long (0·52 mm.) and narrow, 13 times as long as wide at the base. Madagascar (Fig. 18). **E. madagascarensis** n.sp.
— Pegs large, arranged in two rows, aedeagus shorter. 5

5. Claspers 0·38 mm. long. No long seta dorsally near the base, but 2 to 3 short setae. Pegs arranged in 2 rows from the tip to before the middle of the clasper. Pegs of the outer row large, those of the inner row smaller near the tip. A row of hairs begins near the 4th lateral peg and the hairs become longer basally. Aedeagus 0·35 mm. long, 8 times as long as wide at the base. Abdominal ctenidium with 36 teeth. Oriental (Fig. 33). **E. inermis** n.sp.
— A long seta on the dorsal surface of the clasper near the base. 6

6. Clasper short (0·3 mm.). Pegs large, arranged in 2 equal rows from the apical to the basal third of the clasper. Hairs longest near the tip, before the pegs. Aedeagus short (0·23 mm.), 5 times as long as wide at the base and markedly wider apically. Abdominal ctenidium with 28 to 30 teeth. Oriental (Fig. 29). **E. sundaicum** n.sp.

* *Note.* In some specimens of *E. hyrtlii* these spines are very small or absent and the species therefore appears in the key twice.

— Clasper longer (0·43–0·48 mm.), aedeagus longer and narrower. Pegs and hairs on clasper arranged differently. 7

7. Pegs large, arranged in two rows from near the tip to about the middle of the clasper. Pegs of both rows of equal size. Parameres small, triangular with a minute hair. Aedeagus long (0·48 mm.), 8 times as long as wide at the base. Middle East (Fig. 9). **E. hyrtlii** Kol.

— Lateral dorsal row consisting of 5 to 8 large pegs, beginning near the tip. Inner dorsal row consisting of 7 to 8 pegs which are very small or spine like near the tip and larger basally. Parameres large with 3 to 4 small hairs. Aedeagus shorter (0·38 mm.), about 7 times as long as wide at the base. Ethiopian (Fig. 11). **E. africanum** n.sp.

8. Claspers short (0·3 mm.), and broad, tapering only slightly towards a blunt tip. A row of about 7 strong spines which become longer basally at the lateral edge, beginning at the tip. An inner dorsal row of 5 to 6 small spines near the tip. A row of hairs laterally, the longest near the middle of the clasper. Aedeagus 0·35 mm. long, 6 to 7 times as long as wide at the base. Oriental (Fig. 14). **E. latisternum** Schuurm. Stekhoven

— Claspers 0·35 mm. long, wide in the basal half, tapering towards the middle and very narrow in the apical half. Only one dorsal row of 6 to 7 very small spines and a row of hairs laterally. Aedeagus short (0·3 mm.) and very narrow, 9 to 10 times as long as wide. Sternite 5 only slightly convex posteriorly. Oriental (Fig. 20). **E. penthetoris** n.sp.

Females

The characters used are mainly the arrangement of the long setae on the dorsum of the abdomen, the anal sclerite and the genital plates. These are not visible in alcohol material except the ventral plates and the anal sclerite in those species in which the dorsal lip is reduced to lateral flaps, and mounting in balsam is therefore necessary in general.

1. Only 2 long setae near the posterior margin of the spinose area of the dorsum of the abdomen (rarely 3 or 4). Six to 8 long setae at the hindmargin of tergite 6. Ethiopian (Fig. 12). **E. africanum** n.sp.

— Long setae on dorsum of abdomen more numerous. 2

2. Long setae on dorsum arranged in 2 groups, one of shorter setae close to tergite 1 + 2 and a group of longer and stronger setae farther posteriorly. 3

— Only the posterior group of long setae present. 5

3. Anterior group consisting of 8–10 fine setae, arranged in 2 transverse rows. They are not longer than the marginal setae of tergite 1 + 2. Posterior group consisting of two groups of 3 to 5 long setae in the middle of the dorsum. Tergite 6 narrow with 10 to 14 long setae posteriorly. Dorsal genital plate very broad, nearly square. Ventral plates broad, triangular with a row of spines at the dorsal edge which are short pegs near the middle and longer laterally. The 2 rows form an obtuse angle. Anal sclerite absent. Oriental (Figs. 15–17). **E. latisternum** Schuurm. Stekh.

— Setae of the anterior group longer and more numerous. Anal sclerite present. 4

4. Anterior group divided into halves of about 12 setae each. Posterior group also divided into halves of 5 to 7 very long setae in the middle of the dorsum. Tergite 6 elliptical with a row of 8 to 10 long setae posteriorly with a gap in the middle. Dorsal genital plate longer than broad. Ventral plates narrow, triangular with a row of 5 to 6 pegs. Anal sclerite short, broader posteriorly with 2 long setae and several small spines on the surface. Anal segment very short. Middle East (Figs. 4–6). **E. hyrtlii** Kol.

— Arrangement of long setae similar, but both groups not divided into halves and there are one or two pairs of long setae between the anterior and the posterior group. Tergite 6 with only 6 to 8 setae posteriorly. Dorsal genital plate as wide as long. Ventral plates broad, with a row of 6 to 7 spines (not pegs). Anal sclerite long and narrow. Anal segment rounded (Fig. 19). **E. madagascarensis** n.sp.

5. The group of long setae consists of 8 to 10 setae in 2 to 3 transverse rows close together in the middle of the dorsum. Tergite 6 large, roughly rectangular with 2 groups of 3 setae at the sides of the hindmargin. Dorsal genital plate much wider than long. Ventral plates narrow, curved with a row of about 7 long spines which are shorter towards the middle. Ventral plates not covered by the dorsal lip which is reduced to lateral flaps. Anal sclerite short, isolated, with only 2 long setae. Oriental (Figs. 21–23). **E. penthetoris** n.sp.

— The group of long setae stretches from near tergite 1 + 2 to the posterior margin, or nearly so, of the spinose area of the dorsum and consists of 10 to 16 setae. 6

6. Tergite 6 narrow, with a row of 12 to 14 long setae posteriorly. Abdominal ctenidium with 36 spines. Dorsal genital plate very narrow, twice or 3 times as long as wide. Ventral plates triangular, with only 1–3 very small ordinary spines at the inner dorsal corners. A dorsal and a ventral lip present. Anal sclerite small, drop shaped, isolated with only 2 long setae. Oriental (Figs. 34, 35). **E. inermis** n.sp.

— Tergite 6 with a row of only 6 to 8 long setae posteriorly. Abdominal ctenidium with 28 to 33 spines. 7

7. Setae in the middle of the hindmargin of tergite 1 + 2 long. Spiracles of segment 6 lie outside the tergite. The group of 10 to 16 very long setae begins close to tergite 1 + 2 and broadens posteriorly. Dorsal genital plate broadly rounded, as wide as long. Ventral plates triangular, with 2 to 3 thick pegs at the inner dorsal corner and a second row of small spines. Anal sclerite square, with 2 to 3 long setae at the end and several pegs or spines on the surface. Dorsal lip divided into lateral flaps which do not cover the ventral plates. Anal segment short with one group of setae near the anus. Oriental (Figs. 30–32). **E. sundaicum** n.sp.

— Setae in the middle of the hindmargin of tergite 1 + 2 short. Spiracles of segment 6 lie inside the tergite. The group of 8 to 12 moderately long setae begins at a distance from tergite 1 + 2, does not broaden posteriorly and ends usually before the hindmargin of the spinose area. Anal segment rather long, with 2 rows of long setae hollowed out ventrally. Dorsal genital plate rounded, small, fused with the long and narrow anal sclerite into a spoon-shaped structure. Dorsal lip absent. Ventral plates triangular, with a row of setae which are shorter in the middle (Figs. 26–28). **E. philippinensis** Ferris

Dipseliopoda n.g.

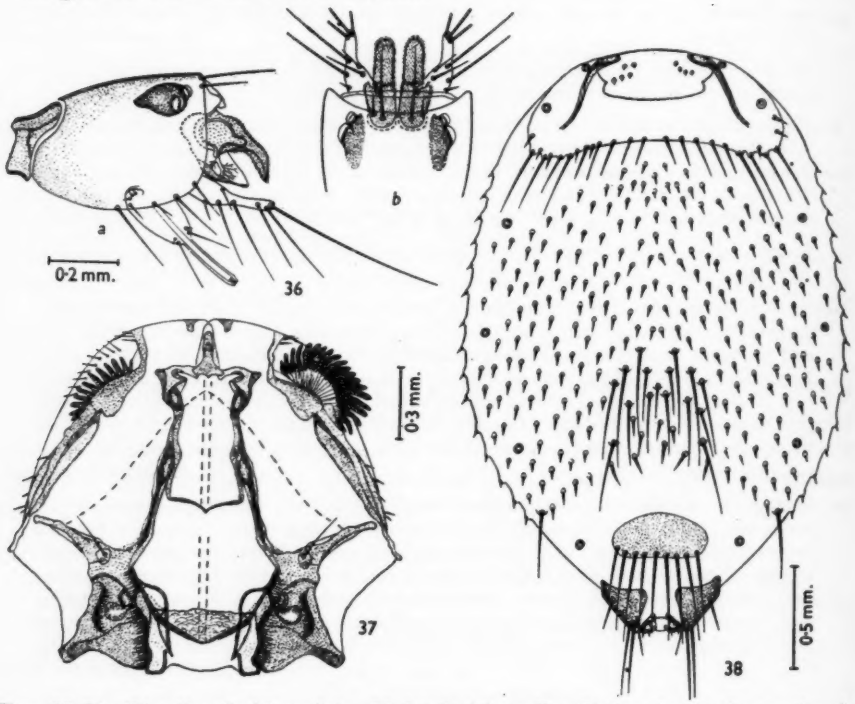
Head moderately compressed laterally. Eyes with two large lenses on a wide, pigmented base, protruding from the surface. Palps of *Eucampsipoda* type, but thick basal part about as long as thin distal part. Labella as long as theka or longer, with 3 small spines at the posterior margin. *Thorax* similar to *Eucampsipoda*. One large and one or two small notopleural setae. Legs similar to *Eucampsipoda*. Tibiae with 2 well-marked rings in the middle. *Abdomen of male* of *Eucampsipoda* type. Sternite 5 with a row of spines at the posterior margin. Anal segment long and more or less conical. *Genitalia*. Phallobase, aedeagus and apodeme of *Eucampsipoda* type. Endophallus without teeth, continuing distally into a long tapering tube or absent. *Abdomen of female* with reduced segmentation. Ventral genital plates divided into halves or partly fused. They bear setae or spines and cover the genital opening from the ventral side as in *Cyclopodia*. Dorsal plate present in one species, absent in others.

The genus is intermediate between *Eucampsipoda* and *Cyclopodia*. It resembles *Eucampsipoda* in the structure of head, thorax, tibiae and male genitalia and *Cyclopodia* in size, in the presence of double eyes, the row of spines on sternite 5 of the male and the form of the genital plates in the female.

1. *Dipseliopoda biannulata* Oldroyd, 1953. (Type species).

Syn. *Cyclopodia biannulata* Oldroyd, 1953.

Length 3·5–4 mm. Colour dark brown.



Figs. 36–38. *Dipseliopoda biannulata*. 36, head: (*a*) profile; (*b*) dorsal. 37, thorax, dorsal. Left ctenidium retracted, right extended. 38, female, abdomen, dorsal.

Head (Fig. 36) moderately compressed laterally with flattened dorsal surface, rather long and rectangular in profile. Two to 4 long setae and 2 short setae laterally at the anterior dorsal margin of the head. Eyes with double, half spherical lenses on a broad, pigmented base. Area surrounding the eyes pigmented, giving the head a spectacled appearance. Palps rather short, with thick base and the anterior thinner part as long as the base. Terminal seta not very long and one or 2 shorter setae close to it. No setae at the thin part of the palp and 2 longer and 2 shorter setae at the base. Labella of labium slightly shorter than the conical theka which has 3 minute spines at the posterior margin.

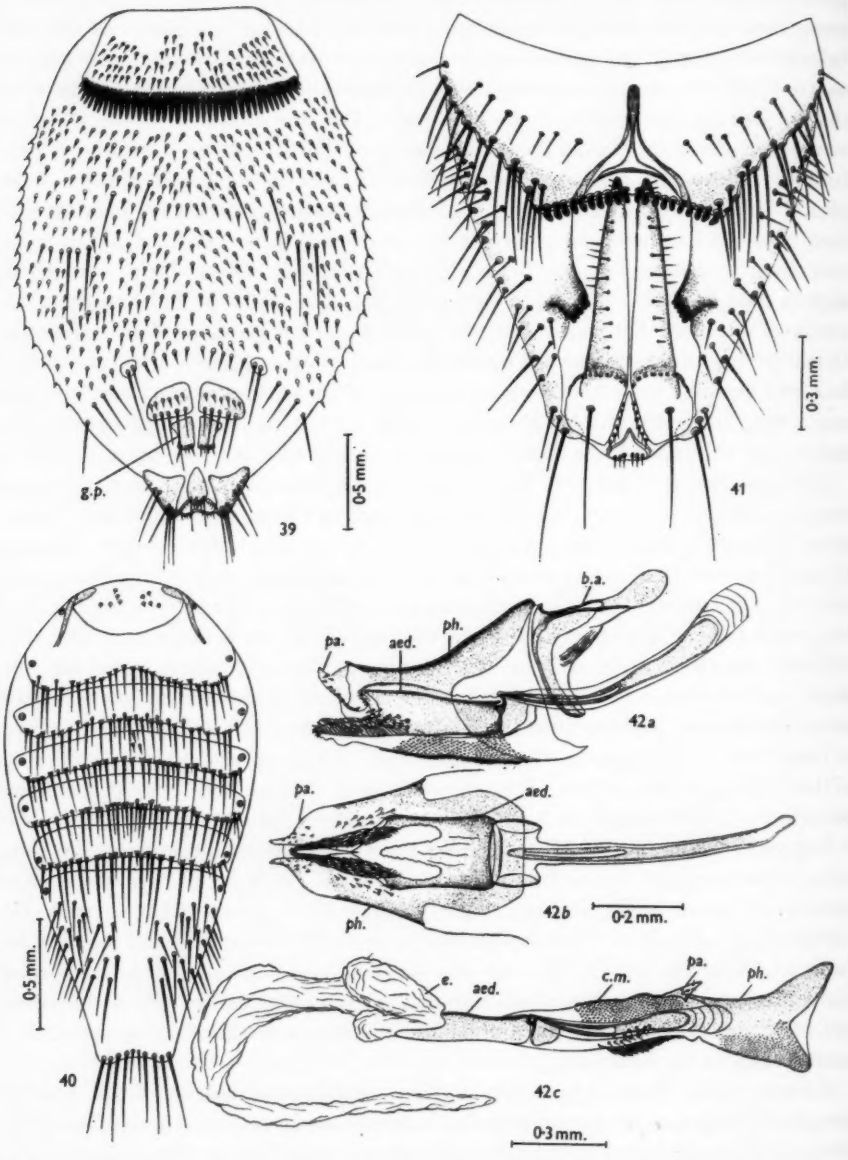
Thorax (Fig. 37) about as long as broad, rounded anteriorly, narrow between the ctenidia which consist of 20 to 22 thick, blunt, curved, striated spines. Sternal median suture narrow, indistinct in the middle. Oblique sutures well marked,

joining median suture at a slightly rounded angle of about 100°. A row of spines along their anterior margin which are nearly as large as the spines of the area behind the sutures. The spines in front of the sutures are very small and present only in 1 to 2 rows along the sutures and in 2 to 3 rows along the anterior margin of the thorax, leaving the middle of the area bare. The row of spines at the posterior ventral margin of the thorax dense, consisting of short setae and a few longer setae laterally. Mesonotal space narrow anteriorly, twice as wide posteriorly. Mesopleural sutures well marked, long and narrow, tapering laterally, bearing near their base one long and one or two short notopleural setae. *Legs.* Anterior coxae very long, conical. Femora long, slightly flattened. Tibiae long, cylindrical, slightly flattened with 2 rings of thinner chitin. The distal ring lies at about the middle of the tibia. Several moderately long setae at the proximal edge of the rings. Dorsal surface of the tibiae with numerous short setae in 3 longitudinal rows. One long seta near the base and two near the apex of tibia 1 and 2 and 2 near the base and 2 near the apex of tibia 3 on the dorsal surface. There is a group of small spines near the base of the dorsal surface of tibia 1 and near the apex of tibia 3.

Abdomen female (Figs. 38, 39). Tergite 1 + 2 triangular with concave hindmargin which bears short, widely spaced setae in the middle and 3 to 4 longer setae alternating with short spines at the lateral corners. Surface bare. Dorsum of abdomen membranous, covered with short spines except in the posterior quarter and with a group of 10 to 14 long setae in 2 or 3 transverse rows in the middle. Two long setae farther posteriorly, near the hindmargin of the spinose area. Tergite 6 elliptical, bare with a row of 9 to 12 long setae at the hindmargin. Anal segment short, conical, with several long setae at the tip of the anal lobes and some shorter setae laterally and posteriorly at the ventral side. Sternite 1 + 2 trapezoid, narrower in front, with a ctenidium of 40 to 44 long, thick, blunt spines and with 3 to 4 rows of short spines on the surface. Post-spiracular sclerite long, with a short spine near sternite 1 + 2. Sternites 3 to 5 membranous, covered with short spines. A row of 4 long setae in the middle of sternite 3 with a wide gap in the middle. Two long setae in the marginal row of the sternite at the sides. The marginal row of sternite 4 consists of spines which are only slightly longer than spines of the surface. The marginal row of sternite 5 consists of shorter spines in the middle and longer setae laterally. Near the sides of the row one long and one short seta stand on a small plate. Sternite 6 divided into two rounded or triangular plates with a transverse row of 5 to 6 short setae in the middle and 3 longer setae alternating with 2 to 3 shorter ones at the hindmargin.

Genital plates. Ventral plate divided into rectangular halves, which bear an irregular double row of 5 to 8 shorter and longer setae at the dorsal margin, covering the genital opening from the ventral side. Dorsal plate absent. Anal sclerite oval, with 2 longer and several shorter setae at the posterior margin.

Abdomen male (Figs. 40, 41). Tergite 1 + 2 with concave hindmargin which bears a row of moderately long setae which are not shorter in the middle as in the female. Surface bare. Tergites 3 to 6 narrow, bare, with rows of moderately long setae at the hindmargin which are longer on tergite 6. Anal segment very large, about as long as tergites 3 to 6 together, conical, covered with short setae except for a stripe



Figs. 39–42. *Dipseliopoda biannulata*. 39, female, abdomen, ventral; *g.p.*, genital plates. 40, male, abdomen, dorsal. 41, male, sternite 5 and genital area. 42, male genitalia: (*a*) resting position, profile; (*b*) resting position, dorsal; (*c*) extended position. *aed.*, aedeagus, *b.a.*, basal arc; *c.m.*, connecting membrane; *e.*, endophallus; *pa.*, paramere; *ph.*, phallobase.

in the middle and along the anterior and posterior margin. Sternite 1 + 2 as in the female. Sternite 3 narrow, with a row of moderately long setae at the hindmargin and 3 rows of short setae on the surface. Sternite 4 similar, but with only one row on the surface which may be double at the sides and interrupted in the middle. Sternite 5 pentagonal, with a row of about 20 short, thick, clubbed spines at the hindmargin, a double row of long setae at the sloping sides and a curved, transverse row of short setae on the surface which is broadly interrupted in the middle. Anal segment ventrally with two broad processes in the middle of the sides which bear 4 to 6 spines, similar to those of sternite 5, at their sharp distal edge.

Genitalia (Figs. 41, 42*a–c*). Basal arc broadly triangular, with a long anterior process and concave posterior margin. Claspers thick and straight, tapering to a dark blunt point. They have a row of fine hairs at the dorsal side, some hairs laterally and a long seta dorsally near the base. Phallobase broad, conical, with fine teeth at the ventral surface. Parameres broad, triangular, with an upturned process and several small hairs. Aedeagus broad, with a long anterior process ventrally and two deep indentations dorsally. Apodeme rather short and broad with a short sleeve around the base. Connecting membrane with two stripes of long, strong teeth ventrally, some long and thin spines at the sides and small teeth dorsally. Endophallus without teeth, with two large dorsal and two smaller ventral lobes continuing into a long membranous tube tapering to a point. The endophallus with the tube is longer than aedeagus and apodeme together.

Hosts and distribution. Ondo, Nigeria from unidentified bat. 4 spec. Types. British Museum (Nat. Hist.). Buea, Nyassoso caves, Cameroons. *Rousettus angolensis*. 52 spec. (Eisentraut). Belgian Congo, Thysville and Banza Nfinda caves. 30 spec. (Leleup). Museum de Congo Belge, Tervueren. Mt. Wago near Blukwa, Lake Albert. *Epomophorus* sp., *Chaerephon faini*. 2 spec. (Fain). Ruwenzori. 2 spec. Rothschild collection, British Museum (Nat. Hist.). A. E. Sudan, Katire near Torit and Talanga forest, Imatong mountains. *Rhinolophus eloquens* or *R. lobatus*. 11 spec. (Hoogstraal, Owen). Chicago Natural History Museum.

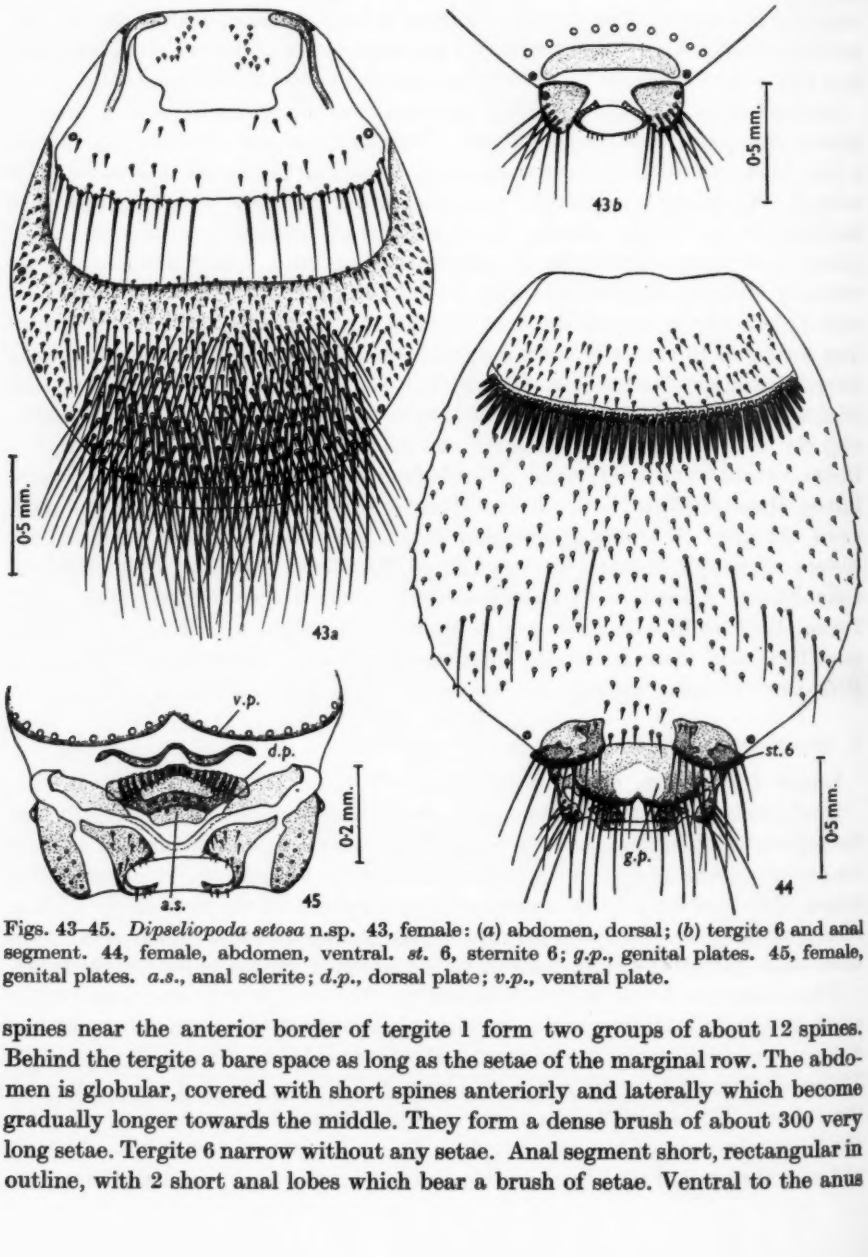
2. *Dipseliopoda setosa* n.sp.

Length 3·5–4·5 mm. Colour dark brown.

Head similar to that of *D. biannulata*, but broader, more rounded dorsally and the pigmentation around the eyes more diffuse. Two long and two shorter setae at the anterior dorsal margin of the head. Palps similar to those of *D. biannulata*, but longer. The tip of the palp has a short point near the base of the terminal seta. Three shorter setae at the thin apical part of the palp and a longer and 2 short setae at the basal thick part of the palp. Labella of the labium 1·5 times as long as the theka.

Thorax also similar to that of *D. biannulata*. The notopleural sutures are farther apart anteriorly and diverge less strongly posteriorly. There is one long and 1 or 2 short notopleural setae. The mesopleural sutures are wider than in *D. biannulata*. The median sternal suture is not interrupted in the middle and of equal width throughout. Tibiae with 2 rings in the middle and with 3 long setae near the base and 2 near the apex on the dorsal surface. The short spines near the base of tibia 1 and near the apex of tibia 3 are smaller and more numerous than in *D. biannulata*.

Abdomen female. (Figs. 43*a*, *b*, 44). Tergite 1 + 2 broadly triangular, with a row of long setae at the hindmargin. Towards the middle the long setae alternate with short spines and there is a gap in the middle of the row. On the surface of the tergite an irregular double or single row of short spines near the marginal row and a few similar spines in a transverse row in the middle of the tergite. The minute



Figs. 43–45. *Dipseliopoda setosa* n.sp. 43, female: (*a*) abdomen, dorsal; (*b*) tergite 6 and anal segment. 44, female, abdomen, ventral. *st.* 6, sternite 6; *g.p.*, genital plates. 45, female, genital plates. *a.s.*, anal sclerite; *d.p.*, dorsal plate; *v.p.*, ventral plate.

spines near the anterior border of tergite 1 form two groups of about 12 spines. Behind the tergite a bare space as long as the setae of the marginal row. The abdomen is globular, covered with short spines anteriorly and laterally which become gradually longer towards the middle. They form a dense brush of about 300 very long setae. Tergite 6 narrow without any setae. Anal segment short, rectangular in outline, with 2 short anal lobes which bear a brush of setae. Ventral to the anus

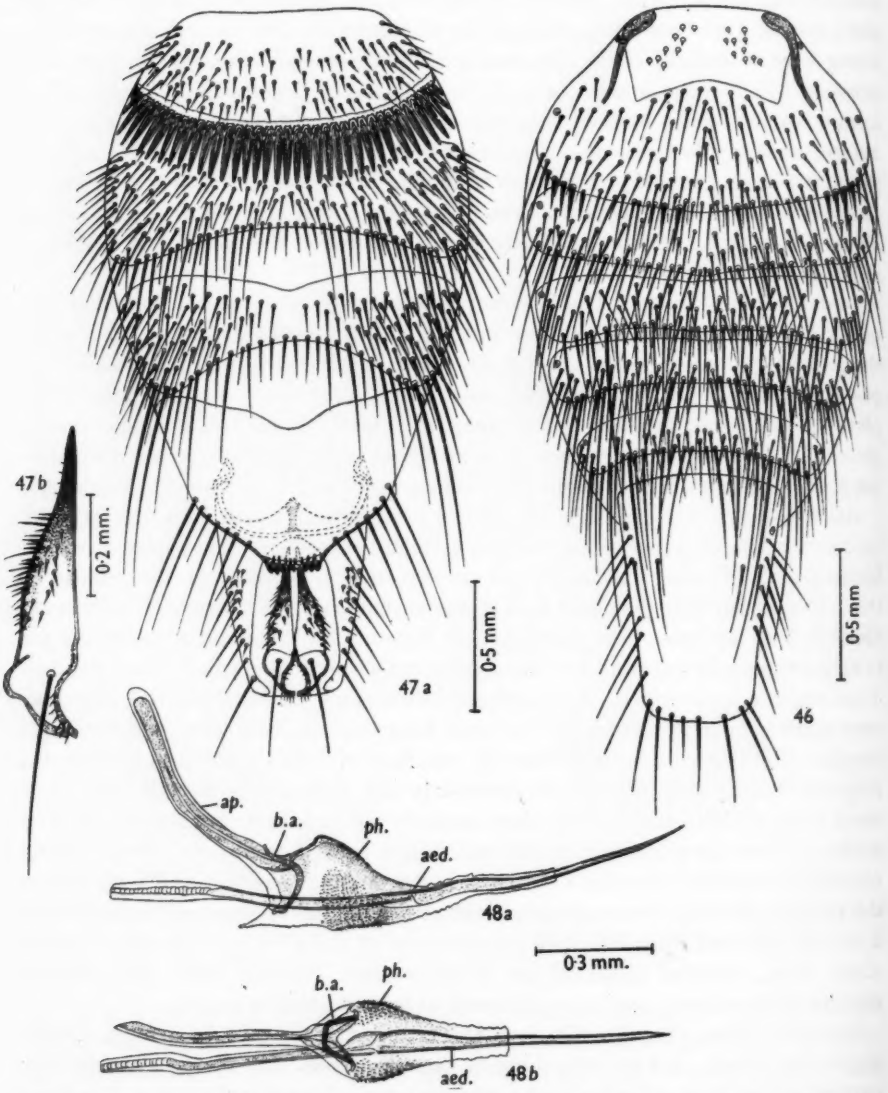
a broad anal sclerite which has a group of about 10 minute spines at the anterior margin. The two sclerites forming the anal frame widen at the ventral side into triangular plates with short spines. Sternite 1 + 2 trapezoid with about 5 rows of short spines in the posterior part and a ctenidium of 38 blunt, thick teeth. Post-spiracular sclerite without a spine in most specimens. In some specimens a short spine present. Ventral side of the abdomen behind sternite 1 + 2 membranous, covered with short spines. Sternite 3 very wide and covered with about 8 rows of short spines. There are 4 long setae in the middle of the penultimate row and 3 to 4 long setae at the sides of the marginal row. These setae and those of the preceding row form a wide curve. Sternite 4 and 5, as indicated by the position of the spiracles, form a common area, covered only with short spines and the border between the 2 sternites cannot be made out. This area is wide laterally and narrow in the middle. Sternite 6 bears 2 roughly elliptical or triangular plates with 3 to 4 long and several short setae at the hindmargin and a row of 4 short spines near the anterior margin. These plates protrude from the body surface. Between the plates 2 to 3 rows of short spines.

Genital plates (Fig. 45). The ventral plates are very wide, broadly rounded and partly fused, leaving an indentation in the middle of the hindmargin. They cover the genital opening from the ventral side. They bear a row of long and short setae posteriorly similar to that of the plates of sternite 6. In the middle of the fused plate a rounded or heart-shaped lighter area. There is a narrow, strip-like dorsal plate with a concave posterior margin which bears a row of short spines. Dorsal to the plate lies a bow-shaped sclerite the connexions of which could not be made out.

Abdomen male (Figs. 46, 47). Tergite 1 + 2 long, triangular, with a dense row of moderately long and short setae and 3 to 4 thick, pointed spines at each side at the hindmargin. In some specimens one or two such spines also in the middle of the hindmargin. The posterior half of the surface (tergite 2) covered with setae about as long as those of the marginal row. Tergites 3 to 6 narrow, with dense rows of long setae posteriorly and 2 to 3 rows of short setae on the surface of tergites 3 to 5 and one row on tergite 6. Anal segment about as long as tergites 3 to 6 together, very narrow, nearly cylindrical, with some long setae at the sides and at the hind-margin. Sternite 1 + 2 as in the female, but shorter, with a ctenidium of 36 teeth. Sternites 3 and 4 narrow, concave posteriorly and with closely standing long and short setae at the sides and only short setae in the middle of the hindmargin. The surface of both sternites is covered with longer setae laterally and shorter setae towards the middle. Sternite 4 with a bare stripe along the anterior margin and in the middle. Sternite 5 triangularly produced in the middle posteriorly and with a row of long and shorter widely spaced setae at the sides and a row of 8 to 10 short, thick, clubbed spines at the truncate apex. Surface bare. The anterior margins of sternites 4 and 5 are indented to form an obtuse angle.

Genitalia (Figs. 47, 48). Claspers thick, tapering sharply to a long, darkly pigmented point. A long seta dorsally near the base. The claspers are thickly covered with spines and setae at the sides and part of the dorsal surface. The inner surface is bare and there are only a few very small hairs at the dark tip. Phallobase conical with small teeth at the sides and ventrally and connected with the aedeagus

by a membrane as in *Eucampsipoda*, but without spines. Basal arc with triangular base and a very long apodeme, which is much longer than in any other species of Nycteribiidae examined. Aedeagus very long and narrow, tapering to a fine point. Endophallus absent. The aedeagus is partly fused with a long apodeme which has a short sleeve around the base. The form of aedeagus and the very long apodeme of the basal arc resemble somewhat the conditions found in some Streblidae e.g. *Nycteribosca*.



Figs. 46–48. *Dipseliopoda setosa* n.sp. 46, male, abdomen, dorsal. 47, male: (*a*) abdomen; (*b*) clasper. 48, male genitalia, partly extended: (*a*) profile; (*b*) dorsal. *aed.*, aedeagus, *b.a.*, basal arc; *ap.*, apodeme of basal arc; *ph.*, phallobase.

HOST AND DISTRIBUTION. Mt. Menengai, Kenya on *Rousettus lanosus*. Type and paratypes in the U.S. National Museum. Paratypes in the Chicago Natural History Museum and the Hebrew University. Ruwenzori, Rothschild collection, British Museum (Nat. Hist.). 1 ♀.

3. *Dipseliopoda arcuata* n.sp.

Length 3–3·5 mm. Colour reddish brown.

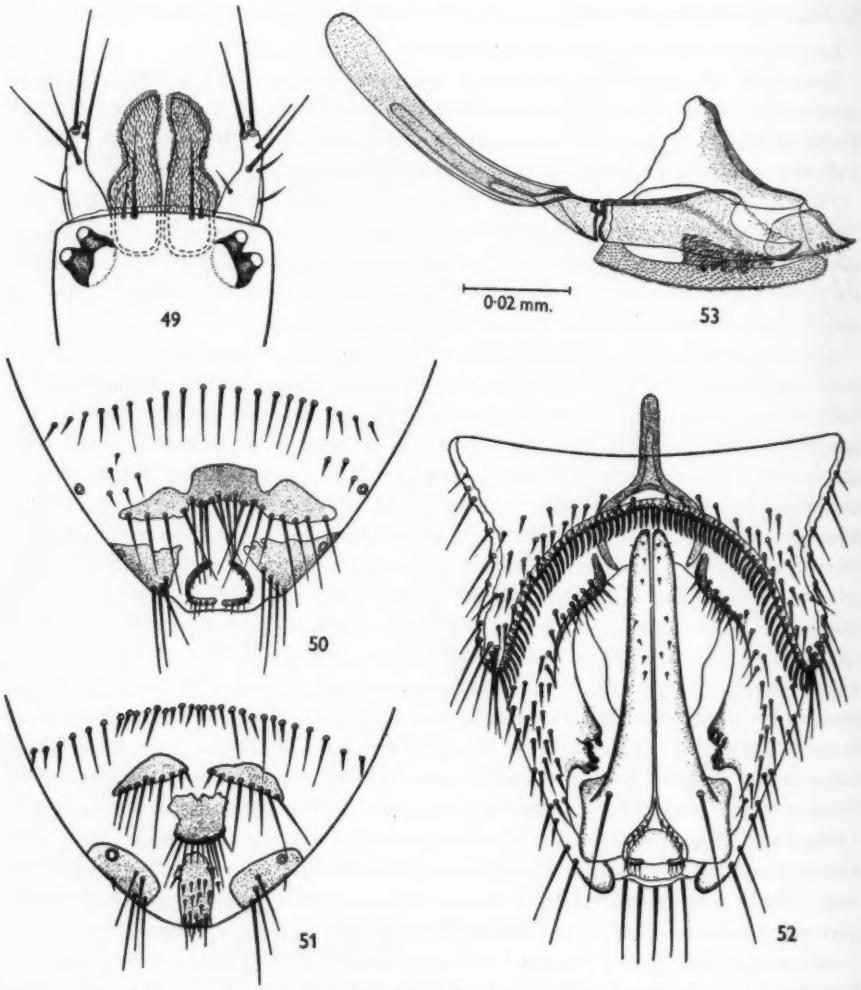
Head (Fig. 49) similar to that of *D. biannulata*. Eyes more prominent, palps shorter and thicker than in *D. biannulata*, but with a similar arrangement of setae. Theka of labium very wide, labella about two-thirds of length of theka. Three to 4 minute spines at each side of the posterior margin of the theka.

Thorax. Dorsal pattern similar to that of *D. biannulata*. There is one long and 2 or 3 short notopleural setae. Median sternal suture indistinct in middle. Legs similar to those of *D. biannulata* except that the long setae on the dorsal surface of the tibiae are shorter, about one-third of the length of the tibia, and the groups of small spines on the dorsal surface of the tibia are absent.

Abdomen female (Figs. 50, 51). Tergite 1 + 2 broadly triangular, with a row of moderately long setae at the posterior margin which are shorter towards the middle. Surface bare, except for a row of short setae in front of the marginal row in the lateral posterior corners. Segments 2 to 5 fused into a membrane, covered with short setae which become gradually longer posteriorly so that those of the last row are about 3 to 4 times as long as those of the anterior rows. There are no long setae on the dorsum as in the other 2 species of the genus. Tergite 6 narrow, tapering laterally and with 2 notches at the anterior margin. There is a row of about 20 long setae at its posterior margin which are more widely spaced laterally than in the middle. In front and laterally to the sclerite there are 2 groups of 4 to 5 short spines. Anal segment short, conical, with a group of 4 to 5 long setae at the tip, which extends into the middle of the segment towards spiracle 7 on the ventral side. Sternite 1 + 2 trapezoid, with a ctenidium of 55 teeth and 2 to 3 rows of short setae in the posterior half of the surface. Sternite 3 to 5 covered with short setae. Their hindmargins marked by some longer setae laterally, particularly on sternite 5. Sternite 6 bare except for 2 narrowly triangular sclerites, each with a row of 6 to 7 longer and shorter setae at the hindmargin. Only one ventral genital plate, with a slightly convex hindmargin which bears a row of about 12 moderately long setae. Anal sclerite long, elliptical, with numerous short setae, mainly in the posterior part and 4 minute spines just outside its basal half in some specimens.

Abdomen male (Fig. 52). Tergite 1 + 2 as in the female. Tergites 3 to 6 very narrow, bare on the surface and with a row of moderately long setae at their posterior margins. The setae of tergite 6 are longer than on the other tergites. The marginal rows are double laterally, particularly on tergites 3 and 4. Anal segment truncate, about half the length of the abdomen, covered with short setae except in the anterior and posterior quarter and with a row of long setae at the posterior margin. Sternite 1 + 2 with a ctenidium of 50 to 52 teeth. Sternites 3 and 4 with a row of moderately long setae posteriorly and 3 rows of short setae on the surface of sternite 3 and an irregular double row on sternite 4. Sternite 5 with 2 broad lateral

processes and between them a wide arc, concave posteriorly, at which stands a dense row of about 65 long, curved, dagger-shaped spines. The lateral processes are covered with short setae which extend in a single row across the sternite. There are some longer setae at the tip of the lateral processes. The ventral side of the



Figs. 49–53. *Dipseliopoda arcuata* n.sp. 49, head, anterior part, dorsal. 50, female, abdomen, posterior part, dorsal. 51, female, abdomen, posterior part, ventral. 52, male, sternite 5 and genital area. 53, male genitalia, profile.

anal segment is also covered with short spines laterally. Anteriorly, there is a strongly chitinized, rounded process with short setae and farther posteriorly a wedge-shaped process, similar to that in *D. biannulata*, each with 4 to 5 short, clubbed spines in 2 groups.

Genitalia (Figs. 52, 53). Claspers long and straight, with a blunt tip, unarmed except for a few minute hairs in the apical half and a long seta dorsally near the base. Basal arc rounded with long anterior process. Phallobase and aedeagus in general like those of *D. biannulata*. Parameres triangular with some small hairs at the ventral edge near the tip. Aedeagus less chitinized than in *D. biannulata*. It could not be determined whether an endophallus is present.

D. arcuata differs from the other 2 species in the absence of long setae on the dorsum of the abdomen and in the presence of a single genital plate in the female and mainly in the shape of sternite 5 in the male.

DISTRIBUTION. 4 ♂♂, 6 ♀♀ Addis Abbaba, Abyssinia, from undetermined bat, Rothschild collection. Type series in the British Museum (Nat. Hist.).

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